University of Alberta

Growth, infection, and iron status of Mexican infants in the first six months of life

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Nutrition and Metabolism

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

Spring 2006

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Your file Votre référence
ISBN: 0-494-13854-8
Our file Notre référence
ISBN: 0-494-13854-8

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Abstract

There is debate surrounding the impact of breastfeeding on growth, micronutrient status, and infection from 0-6 months. A prospective study in Guadalajara, Mexico was undertaken to examine feeding, growth, infection, and iron stores in 154 infants born to low-income women. Monthly, weight and length were measured, and mothers reported symptoms of infant illness and feeding method. Infants were grouped according to feeding mode during 0-4 months with 35% primarily breastfed (BF), 25% partially breastfed (PBF), and 33% formula fed (FF). No differences were observed among feeding groups in growth or incidence of upper respiratory infections. At six months, the odds of iron deficiency were 5.4 among BF infants. However, the odds of a gastrointestinal infection (GI) were 2.1 among not BF infants (PBF, FF combined) (20 vs. 35%, $p<.05$). Thus, BF infants grew adequately and were protected against GI during early infancy, but had reduced iron stores at six months.

Acknowledgements

I would like to begin by thanking the numerous funding sources for this project: University of Alberta International, Juan I Menchaca Hospital, and Dr. Noreen Willow's start-up funds and establishment grant from Alberta Heritage Foundation for Medical Research.

The thesis would not have been possible without the participation of our the mothers in the study. Their kind-hearted nature and their devotion to the well-being of their infants was the driving force to continue with research in this subject area. Thank you for allowing me to share in the care of your infants. Watching your babies grow and develop was a wonderful experience in which I was richly rewarded with numerous baby smiles.

I would like to express my sincere appreciation and admiration to Dr. Edgar Vasquez and Dr. Enrique Romero whose help in coordinating the project in Guadalajara was invaluable. Also, I would like to thank the study pediatrician, Dr. Marta Luna, and nursing and laboratory staff at the Juan I Menchaca Hospital. In particular, I would like to express gratitude to my research assistant, Jissela Alfaro, who worked on the project from the beginning, aided in solving logistic issues, and when I left, took over as the study coordinator.

Also, a very special warm-hearted thank-you to Noreen Willows, my supervisor: Noreen, you believed in my abilities and supported me unconditionally. Thank you for being my teacher and my mentor. I would like to thank my committee members, Dr. Linda Casey and Dr. Catherine Field, for their input on this thesis. Dr. Edward Frongillo, thank-you for your invaluable help on the statistical analyses, and for your input on the study design and questionnaires.

To my family, friends, and Arnold who have supported me throughout this journey. Your encouragement was necessary to see me this through. Thank you for walking with me and for allowing me to share this life experience with you all.

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Abbreviations

AAP	American Academy of Pediatrics
BMI	Body mass index
BFHI	Baby Friendly Hospital Initiative
BF	Breastfed
CRP	C-reactive protein
CS	Cesarean section
EBF	Exclusively breastfed
ENN	Encuesta Nacional de Nutrición
ESR	Erythrocyte sedimentation rate
FAO	Food and Agriculture Organization
FF	Formula fed
GI	Gastrointestinal infections
GMA	Greater metropolitan area
Hb	Hemoglobin
Hct	Hematocrit
IMR	Infant mortality rate
IUGR	Intrauterine growth retardation
LA	Length for age
LAZ	Length for age Z-score
LBW	Low birth weight
MCV	Mean corpuscular volume
NCHS	National Center for Health Statistics
PBF	Partially breastfed
SD	Standard deviations
SES	Socioeconomic status
UNICEF	United Nations Children's Fund
URTI	Upper respiratory tract infections
WA	Weight for age
WAZ	Weight for age Z-score
WL	Weight for length

WLZ Weight for length Z-score

WHO World Health Organization

1. Introduction

1.1. Background

Breastfeeding is recognized as the optimal method of infant feeding. Breast milk provides the nutrition for infant growth and development, confers immunity from infections (Hamosh 2001), and a mother's breast serves a physical barrier to contaminated foods or liquids. Nutrition in early infancy has been demonstrated to affect growth and cognitive development in childhood and early adolescence (Brown and Begin 1993). Also, breastfeeding positively affects short and long-term nutritional and health status of the mother: decreases post-partum bleeding, induces lactation amenorrhea, enhances loss of weight gained in pregnancy, and decreases the risks of breast and ovarian cancer (AAP 2005). Ultimately, it is the mother's choice to breastfeed her infant and that choice is influenced by cultural beliefs, social environment and breastfeeding problems (PAHO 2002). The challenge lies in promoting and achieving the exclusivity of breast milk.

The relationship between infant feeding mode, growth, and morbidity has been extensively researched, yet there is still much debate around the adequacy of exclusive breastfeeding and its optimal duration on infant health outcomes (Butte et al. 2002; Kramer and Kakuma 2001). In an expert consultation (WHO 2001; Habicht 2004) for the most recent WHO public health recommendation on breastfeeding, the researchers agreed that recommending exclusive breastfeeding to 6 months of age outweighed the risks of inadequate iron status and inadequate growth at six months in some infants. In order to improve the precision estimates and applicability of exclusive breastfeeding from 0-6 months of age, the committee did acknowledge the need for research in the following areas: comparison of exclusive/predominant breastfeeding to partial breastfeeding from 0-4 months versus 0- 6 months on growth status, micronutrient status, and diarrheal morbidity at six months of age and beyond.

1.2. Research questions, hypotheses, and objectives

A study in Guadalajara, Mexico was undertaken to answer two research questions:

1. How do infant feeding practices influence infant growth, and cumulative incidence of gastrointestinal and upper respiratory tract infections from birth to six months, and iron status at 6 months of age?
2. What are the trade-offs between feeding practices on infection during the first six months and iron status at 6 months of age?

Hypothesis: Breastfed infants will differ from formula fed infants in weight gain, cumulative incidence of gastrointestinal infection, and iron status. No differences are expected in length or cumulative incidence of respiratory infections among feeding groups.

Objectives:

1. For all the infants in the cohort, describe growth from birth to six months, cumulative incidence of infection, and iron status at six months.

2. Explore the relationships between infant feeding practices (predictor variable), and outcome variables while controlling for significant covariates.

   a) Compare changes in growth, cumulative incidence of infection and iron status across infant feeding groups.

2. Review of the literature

This literature review discusses current and selected topics related to healthy, normal birth weight, full-term infants. Topics reviewed are infant feeding, growth, gastrointestinal and upper respiratory tract infections, and iron status. Also discussed are the assessment and methodologies of these topics. When applicable, focus will be placed on Mexican and Latin America research studies.

2.1. Mexican Context

Mexico has developed an effective nutrition surveillance system to assess the nutrition practices, and health outcomes of women and young children. The National Nutrition Survey or ENN (acronym in Spanish) is a probabilistic survey representative of

the Mexican population. The households are selected using cluster sampling. Households included in the survey are those with one or more of the following groups under study: women of childbearing age (12-49 years), children less than 5 years of age, and school aged children (5-11 years of age) (Resano-Perez et al. 1999). Normally, the surveys are undertaken every 6 years.

Mexican data on the sociodemographic characteristics of women, health of women and infants, and infant feeding practices from the most recent survey, ENN-1999 (Rivera-Dommarco et al. 2001), are presented henceforth unless otherwise indicated.

A. Demographic and health information of Mexican women and infants

Women of childbearing age Mexico's population of 103 million grows at a rate of 1.85% per year (SRE 2000). About 44% of the population is under the age of 18 (UNICEF 2005). Approximately 75% of the population lives in urban areas and the average population density is 50 people per square kilometer (SRE 2000). Particularly, 93.5% of women of child bearing age (12-49 years) are literate, and over 50% have completed 9 years of basic schooling. Fifty percent are homemakers, 20% are students, and 30% are employed in remunerated work. Parity (as a function of total live births) is 2.2 children per woman (INEGI 2000).

Prevalence of anemia, as measured by hemoglobin concentration <110 g/L, during pregnancy is high (26.4%). This is a concern because anemia is considered a risk factor for low birth weight (Rivera-Dommarco et al. 2001). The diet of Mexican women is poor in iron rich foods and is thought to be the major contributor to anemia in pregnancy, as Mexico does not have significant incidence of malaria or endemic hookworm infestation.

Infants The latest statistics reveal that 9% of Mexican infants are born low birth weight (<2500g) (UNICEF 2005). IMR (rate of death in children under 1 year of age) is estimated at 23 deaths/1000 live births (UNICEF 2005), which is a slightly less than the IMR for Latin America and the Caribbean of 27/1000 live births (UNICEF 2005). In Mexico, using the WHO criteria of 2 standard deviations below the reference mean, infants 0-11 months old 3.4% were underweight (weight for age), 8.2% were stunted (length for age), and 2.5% were wasted (weight for length). Stunting prevalence increases dramatically to 21.6% in infants 12-24 months of age. In Mexico, stunting and

underweight in children remains a public health issue; whereas, wasting is not a public health concern, with the exception of some specific groups such as rural, poor children.

Overall, prevalence of anemia (Hb <110 g/L) in children under the age of five is 27.2%. Specifically, 13% of infants 6-11 months old are anemic. The highest prevalence (48%) of anemia occurs in the second year of life, while 33% of children 2-3 years old are anemic. In children under five, intake of iron and vitamin C is 64% of recommended values, vitamin A is 86% of recommended values, and total energy intake is 74% of recommended values. However, the children's diet meets 100% of recommended daily intake for folic acid and protein. The cause of anemia in infants and toddlers is iron deficiency secondary to poor intake (Rivera-Dommarco et al. 2001).

B. Feeding practices and infant survival in Latin America

It has been suggested that breastfeeding be considered as necessary for child survival as immunization (Lancet 1994). For instance, UNICEF (Statistics 2000b) estimates that 1.3 million lives could be saved if infants were exclusively breastfed until six months of age. Betran and colleagues (2001), using national data on infant mortality, estimated that 55% of the diarrheal and respiratory deaths, and 13.9% of all-cause mortality in Latin America are preventable through exclusive breastfeeding. Most of the reduction in mortality would occur in infants 0-3 months of age. Partial breastfeeding from 4-11 months of age would prevent 37% of deaths attributed to diarrhea (Betran et al. 2001). Betran et al. (2001) also reported that successful breastfeeding promotion, to increase breastfeeding rates by 25%, would save 13 000 lives each year. In a similar study of pooled mortality and breastfeeding data from various less developed countries, deaths from diarrheal illness was six times more common in non-breastfed than breastfed infants (WHO Collaborative Study Team 2000). Breastfeeding was also protective against deaths caused by respiratory infections (odds ratio: 2.4) (WHO Collaborative Study Team 2000).

C. Current feeding practices in Mexico

In the last ENN, 92% of mothers reported providing some breast milk to their infants since birth (ever-breastfed) (Gonzalez-Cossio et al. 2003). These high ever breastfed rates may be explained, in part, by the large number of Baby Friendly

Hospitals. The BFHI was launched by the WHO in 1991 (UNICEF 2002). The Baby Friendly Hospitals provide an environment that promotes the exclusivity of breast milk (CHD 1998) for an early and successful initiation of breastfeeding (UNICEF 2002). Breastfeeding success has been linked to timely initiation of breastfeeding after birth (within two hours post-partum) (Guerrero et al. 1999). Mexico has 692 certified Baby Friendly Hospitals (UNICEF 2002). Nationally, median duration of any breastfeeding is nine months. The number of children exclusively breastfed for less than four and six months is 25.7% and 20.3%, respectively. In Guadalajara, the rates of exclusive breastfeeding at one, three, and six months are 51%, 20.2%, and 20.4%, respectively (Santos-Torres 1990).

Demographics of infant feeding Although the BFHI is successful at helping mothers initiate and increase the mean duration of breastfeeding (Kramer et al. 2001), breastfeeding is influenced still by other complex biological, socioeconomic and cultural factors that researchers do not fully understand (Butte et al.2002). For example, infants most likely to be exclusively breastfed are girls, and smaller babies (González-Cossio et al. 2003). At the national level, exclusive breastfeeding is higher among women of lower SES, who are unemployed, have low levels of education, have no health care insurance, belong to a rural community, or are of indigenous ethnicity (González-Cossio et al. 2003). Researchers working in rural communities have found that younger women or those with previous breastfeeding experience practice exclusive breastfeeding (Flores et al. 2005). Women less likely to initiate exclusive breastfeeding from birth are older or work outside the home (Flores et al. 2005). In Guadalajara, Santos-Torres and colleagues (1990) reported that exclusive breastfeeding was less common in mothers with more education, higher SES status, or those who were primipara. Age, in this group of women, did not significantly influence breastfeeding practices (Santos-Torres et al. 1990).

Cultural practices of infant feeding Exclusive breastfeeding is not commonplace in Mexico because it is customary to introduce teas or water to infants in the first month of life. Guerrero et al. (1999) reported that 63% of infants were given tea or water by 20 days of age. Mennela et al. (2005) also reported that one third of infants had received tea, water, or formula by the first week of life. Water and teas commonly given during infancy are for medicinal purposes (Mennela et al. 2005). Because of mothers' perceived

milk insufficiency, there is a tendency to terminate breastfeeding before three months (Santos-Torres et al. 1990). In Mexico, food is usually introduced by 4 months (Guerrero et al. 1999) and common first foods are fruits followed by vegetables (Mennela et al. 2005).

2.2. Infant feeding from 0-6 months of age

A. Nutritional composition of human milk

Colostrum, a thin fluid, is the first liquid secreted from the mammary gland hours after birth and mainly consists of proteins and lactose (Berne and Levey 2000). Milk production is usually established between 7-10 days post-partum (Berne and Levey 2000). Factors that influence the composition of human milk include maternal nutrition and stage of lactation (Picciano 1998). For some nutrients, diurnal variations, or variable concentrations between hind and fore milk are common (Picciano 1998). Nevertheless, breast milk is the most suitable form of nutrition due to its concentration and quality of proteins, lipids, carbohydrates, vitamins, and minerals (Picciano 1998).

Macronutrients Human milk is 1% w/w protein, 7% w/w lactose, and 3.5% w/w fat (Berne and Levey 2000). Protein concentration of human milk is high in the early stages (lactogenesis I) of lactation (15.8g/L) and progressively diminishes to 8-9 g/L in mature milk (Picciano 1998). The type of protein (40% casein and 60% whey protein) (Fomon 1993) varies with maternal nutritional status, but the impact on infant nutrition remains unknown (Picciano 1998). Whey protein consists of lactoalbumin, lactoferrin, and immunoglobulins (Fomon 1993). The protein fraction of human milk also contains free amino acids, and nucleic acids all of which are essential for infant growth, and development of the immune system (Picciano 1998).

Lipids, the most variable component of human milk, are directly controlled by the maternal diet and nutritional status (Picciano 1998; Butte et al. 2002). Malnourished women produce only 60% of the milk lipid content of not malnourished women (Brown et al.1986). Long chain trigylcerides comprise 97- 98% of the lipid fraction; triglycerides provide most of the energy found in breast milk (Picciano 1998). Fat has marked diurnal variation (Picciano 1998). Fat content is lowest in the morning, highest at midday, and then steadily declines (Picciano 1998). Breast milk also contains cholesterol, and fatty

acids such as linoleic acid, a-linolenic acid, arachidonic acid (AA), and docosahexaenoic acid (DHA) (Picciano 1998). DHA is necessary for brain and visual development (Innis 2004). AA and its metabolites are essential for cell signaling and division; more recently AA has been linked to infant growth (Innis 2004).

Lactose, the disaccharide in breast milk (6-7g/100mL), is the major constituent of the macronutrients (Picciano 1998). Nucleotides and oligosaccharides are only minor constituents of human milk but play an important role in infant development (Picciano 1998).

Vitamins The nutritional status of the mother affects the concentration of vitamins in breast milk (Butte et al. 2002; Picciano 1998). Vitamin A concentration decreases in breast milk as lactation continues. Women with sub-optimal levels of vitamin A will have lower levels in their milk; however, maternal vitamin A supplementation improves breast milk concentration of vitamin A (Butte et al. 2002). The bioavailability of preformed vitamin A from human milk is about 90% (Butte et al. 2002). Adequate levels of vitamin A are necessary for immune function of the infant (Butte et al. 2002). Human milk in combination with adequate exposure to sunlight meets an infant's vitamin D requirements (Butte et al. 2002). Maternal intake of vitamin D does not affect milk concentrations (Butte et al. 2002). Vitamin K concentration in milk is sufficient to prevent vitamin K deficiency in infants who have received intramuscular vitamin K at birth (Fomon 1993). Vitamin K concentrations are greater in colostrum than mature milk (Fomon 1993). Vitamin E is highest in colostrum and found mostly in the form of α-tocopherol (Picciano 1998).

Breast milk concentration of vitamin C in well-nourished women is about 100 mg/L (Picciano 1998). Thiamine levels increase as breast milk matures whereas both riboflavin and vitamin B_6 are affected by a mother's intake and decline progressively throughout the lactation process (Picciano 1998). The length of gestation affects endowment of vitamin B_6 at birth so preterm infants are susceptible to a B_6 deficiency (Butte et al. 2002). Vitamin B_6 deficiency can negatively influence growth and neurological development (Butte et al. 2002). Vitamin B_{12} and folate are bound to whey protein. Maternal diet does not seem to affect milk folate concentrations even in mothers with reduced folate stores (Picciano 1998).

Minerals The mineral content of breast milk does not seem to be correlated to the serum levels of the mother (Picciano 1998). Mature human milk contains sufficient amounts of calcium (250 mg/L; Fomon 1993), magnesium (28-35 mg/L; Fomon 1993), and phosphorous (120-160 mg/L; Fomon 1993) to support bone growth and remodeling (Picciano 1998). Electrolyte concentration is tightly regulated in breast milk (Picciano 1998).

Iron and zinc in human milk for the most part have a high bioavailability (Picciano 1998) even though their overall concentration is low. Zinc is bound to citrate, a promoter of zinc absorption (Fomon 1993) and consequently an infant absorbs about 40% of the zinc in human milk (Fomon 1993). Iron, which is bound to lactoferrin, is highly available (50% absorption rate) (Fomon et al. 2000) and it is absorbed 4-5 times more efficiently than iron in cow's milk (Picciano 1998). Human milk alone cannot meet the iron and zinc requirements of the breastfed infant beyond 6 months of age (Butte et al. 2002) and in some breastfed infants, iron deficiency may be a concern before six months of age (Fomon 2001). Maternal intake or endogenous stores do not affect zinc or iron, concentrations in the breast milk (Picciano 1998). Both iron and zinc are associated with the development of the immune system (Butte et al. 2002). Zinc has been linked to infants' linear growth (Butte et al. 2002). Iron stabilize during lactation to about 0.3 mg/L whereas zinc concentration declines (Picciano 1998). Zinc and iron are highest in the colostrum (Picciano 1998).

Non-nutritive components Hamosh et al. (2001) in her review discusses the components in breast milk that promote functional development of the infant. Enzymes present in breast milk (bile salt dependent lipase and amylase) aid in fat and carbohydrate digestion until the neonatal pancreas matures at around 2 months of age. Cassmorphins, products of casein digestion, are opoid antagonists, which have been shown to modulate behaviour and the immune system. Breast milk contains glycosylated or phosphorylated growth hormones and may impact infant growth and development in the short and long term. For example, prolactin acts immediately on T-lymphocytes to aid mitosis, but prolactin also modulates the neuroendocrine system later in life (Hamosh 2001).

B. Energy requirements for adequate growth

Infants 0-6 months of age require 108 kcal/kg of body weight to meet growth and developmental needs (ADA/DC 2003). Both human milk and infant formula provide on average 70 kcal/100 mL (ADA/DC 2003). Breast milk production and consumption increases as the infant grows. Thus, an infant at 1 month of age consumes on average 699g/d, whereas an infant at 6 months of age consumes on average 854 g/day (Butte et al. 2002). An infant who feeds adequately will usually feed at the breast, on average, 8-12 times per day (ADA/DC 2003). Exclusive breastfeeding for six months supports adequate growth and functional development if the maternal diet is adequate and sufficient quantity of breast milk is produced (Picciano 1998).

C. Infant feeding recommendations

In 2001, the WHO changed its recommendation of exclusive breastfeeding from 0-4 months to exclusive breastfeeding from 0-6 with continued breastfeeding to two years of age and beyond (WHO 2001b). The WHO (1991a) defines exclusive breastfeeding as no other food or drink, including water. EBF allows for medicine drops and syrups to be provided to the infant if they have been prescribed by a physician. The predominant breastfeeding definition allows for liquids such as water, teas, and juices to be given to the infant but breast milk remains the predominant source of nutrition (WHO 1991a).

North American professional associations have only recently changed their recommendation to exclusively breastfeeding all healthy term infants for six months (AAP 2005; Health Canada 2004). These associations agree that breastfeeding should be regarded as the normative standard to assess growth, health, and development. Breastfeeding contributes to eating in moderation (Fomon 2001), which may in part modulate overweight and obesity in infancy (AAP 2005). Epidemiological evidence suggests that breastfeeding may also reduce the risk of overweight and obesity in childhood and adulthood (AAP 2005). Research demonstrates reduced incidence and/or severity of gastrointestinal and upper respiratory tract infection in breastfed infants. Because of these positive health outcomes, breastfeeding can also reduce acute care costs (AAP 2005).

The current 'globalized' recommendations for exclusive breastfeeding, and consequently for introduction of complementary foods, does not allow for individual

needs of infants (Lutter 2000). The current breastfeeding recommendation poses a challenge to biology, science and public health (Lutter 2000) because there is no evidence to suggest exactly when infants nutrient stores are depleted (Butte et al. 2002). Current data indicate that the older the infant, the more likely the risk of a nutritional deficiency (Butte et al. 2002). Some infants may require a weaning diet at an earlier time than 6 months secondary to signs of growth faltering or nutritional deficiencies. Lutter (2000) suggests recommending a mean age with upper and lower bounds to accommodate those infants that require complementary foods early, and infants that can continue breastfeeding exclusively past the recommended period without impairing growth and development.

(1) Weanling's dilemma

The weanling's dilemma occurs when an infant is weaned from breast milk to solid food that may be nutritionally inadequate, contaminated, or both (Brown and Begin 1993). The weanling's dilemma is the compromise between early introduction of weaning foods and its inappropriate delay (Garza et al. 1994). In other words, infant growth may begin to falter if inadequate food is introduced early and will falter if exclusive breastfeeding is continued beyond its ability to sustain growth. Inappropriate weaning practices lead to nutritional stunting (LAZ 2 SD below the mean). Brown and Begin (1993) view the weanling's dilemma as a serious issue for infant survival and morbidity in developing nations.

D. Challenges in meeting feeding recommendations

Generally, factors associated with the duration of exclusive breastfeeding are medical advice, resumption of work or school, family advice, and marketing of infant formulas (Fulhan et al. 2003). Guerrero et al. (1999) found that feeding advice from physicians and other caregivers, such as the maternal and paternal grandmother of the infant, greatly influenced feeding practices. A mother's own knowledge about breastfeeding, previous success or failure in breastfeeding, and perceived ideas of personal health or of their infant's, affect breastfeeding decisions (Pelto 2000). Less important factors are cost, comfort, or a husband's opinion (Guerrero et al. 1999).

Biological determinants such as an infant's size, sex, growth rate, appetite, and a mother's lactation capacity also influence breastfeeding (Butte et al. 2002). Furthermore, pacifiers, cigarette smoking, and problems with breastfeeding are persistent barriers to exclusive breastfeeding (Fulhan et al. 2003).

It is important to emphasize that infant feeding is not a static behaviour and feeding practices may vary on a daily basis (Guerrero et al. 1999). It is not uncommon for mothers to abruptly suspend and then resume exclusive breastfeeding or breastfeeding (Guerrero et al. 1999). Mexican women will introduce formula in the first week of life and return to breastfeeding thereafter once successful lactation has been established (Macias-Carillo et al. 2005).

In Mexico, there is an overall positive attitude towards breastfeeding but no real belief that EBF is indispensable for the optimal health of the infant (Guerrero et al. 1999; Mennella et al. 2005). Cultural beliefs play a significant role in feeding decisions. For example, maternal food taboos during lactation have been associated with the decision to complement breastfeeding (Santos-Torres 2003). Folk illness such as *coraje* (anger), *susto* (fright), *mal de estomago* (upset stomach), are treated with teas (Guerrero et al. 1999). It is common to give an infant tea for colic, earaches, to treat a cold, for hydration, to reduce crying, or to relieve constipation (WHO 1997).

Pelto (2000) provides an anthropology perspective on the complexities of infant feeding. Collecting socioeconomic status (SES) data, although strongly associated with infant feeding behaviours, provides a description but no explanation as to why behaviours are expressed. Infant feeding practices are transmitted through generations in a process known as enculturation. However, positive attitudes do not necessarily translate into practice. The multiple roles of a woman within society such as wife, daughter-in-law, and income earner often lack synergism with the role of motherhood. Breastfeeding is a choice and not an un-thoughtful act. As such, the merits and shortcomings of breastfeeding are used to make the decision and likely alternatives to that choice. Feeding choice is a process that is modified over time by a myriad of factors (Pelto 2000).

2.3. Growth in infants

Growth is regarded as a proxy for child health because children who grow well are generally healthy (Cole 2002). To assess if the growth of a child is adequate, an infant's weight and length are plotted on growth charts (WHO/NCHS 1977). Growth charts are based on the observed growth of a healthy population of infants living in environments that are conducive to reaching maximal growth potential (WHO 1995b). This population is often regarded as a reference population because at present there are no true standards for growth (Keller 1986).

Growth assessment requires serial measurements since growth is defined as increased size over time (WHO 1995a). Growth velocity is the rate of change in size over time; whereas attained size refers to the distance the infant has travelled from birth (Cole 2002). In the first year of life, infant growth can occur quickly (crossing percentiles upwards) or more slowly (crossing percentiles downward) (Cole 2002). Longitudinally, growth failure/faltering occurs when an infant's weight or length crosses two major percentile lines downward (ie: 75^{th}, 50^{th}, or 25^{th} percentiles) (McMeans 2005). Thus infants who are growing well demonstrate positive, upward growth on the curves.

Weight and length measurement plotted on growth charts are used to derive anthropometric indices: WA, WL, and LA indices (WHO 1995a). Generally, a deficit (2 SD below the reference mean) in one or more of these indices is evidence of malnutrition; however, malnutrition may be a result of infectious or parasitic disease as well as dietary inadequacy (WHO 1995a). Infants living in hostile environments (poor, unhygienic) could have a combination of these factors, thus, making it difficult to assess the cause of growth impairment (WHO 1995a). This is an important consideration in that health practitioners assess growth, lactation adequacy, and advise on timing of introduction of complementary foods based on anthropometric indices and their deviations from the mean of the reference population (de Onis and Onyango 2003).

The impact of impaired infant growth extends beyond the first year of life. For example, childhood stunting influences attained adult size (WHO 1995a). Smaller adults have a lower work capacity in manual labour. Furthermore, maternal height is strongly associated with birth weight (WHO 1995a). Stunting in women may be one of the

important transcendental factors that impact the health of future generations (WHO 1995a).

A. Growth curves

Evaluation of growth requires an appropriate reference. In practice, growth curves serve more as a standard (how healthy infants should grow) than as a reference (how infants grow). A standard is the concept of a norm or a target that should be reached.

The WHO/NCHS reference curves were developed in 1975, and combined the data of 4 distinct American surveys (WHO 1995b). One of the data sources was the Fels Research Institute Longitudinal Study from 1929-1975 in Yellow Springs Ohio (WHO 1995b). From 1929-1975, infant dietary recommendations condoned the introduction of solid foods in the first month of life and formula feeding was the norm (Whitehead and Paul 2000). The Fels Study population was homogenous in genetics, geography, and socioeconomic status which limit its ability to serve as a reference. Despite standardized anthropometric procedures, the weight and length measurements were done too infrequently at 1, 3, 6, 9, 12, and 18 months (WHO 1995b). Inadequate and outdated curve fitting techniques were used and not all infants were followed from birth (Victora et al. 1998). Furthermore, The WHO/NCHS weight curves are positively skewed indicating a high degree of obesity in the reference population (Victora et al. 1998).

In 2000, the Center for Disease Control released a revised version of the old WHO/NCHS charts (Fulhan et al. 2003). Improved smoothing techniques were used and more breastfed infants were included (Fulhan et al. 2003); although, very few infants were breastfed for more than a few months (de Onis and Onyango 2003). Five different surveys were used to develop the infancy section (de Onis and Onyango 2003). The new CDC charts were derived from the Fels data set along with the US vital statistics from Missouri and Wisconsin, and the Pediatric Nutrition Surveillance system for infants 0-6 months (de Onis and Onyango 2003).

Diagnosing growth faltering remains the main issue surrounding the current CDC and WHO/NCHS growth charts (de Onis and Onyango 2003). On both curves, however, BF infants appear to falter at around 2 months of age. This has serious implications for field workers that use WA to diagnose growth faltering. The CDC curves systematically classify more BF infants as wasted and underweight, and less as stunted (de Onis and

Onyango 2003). For these reasons, both the CDC and WHO/NCHS curves remain inadequate to assess the growth trajectories of healthy BF infants (de Onis and Onyango 2003). The technical problems of the curves do not allow for a proper assessment as to whether differences in growth are due to feeding patterns or inadequacies of the curves (Dewey 1998c).

To solve the problem of inadequate and out-of-date growth curves, the WHO completed a multi-country trial and developed new curves based on the growth of breastfed infants living in healthy environments (Dewey 1998b). The reference population was able to follow the WHO recommendations for exclusive breastfeeding; was ethnically and racially diverse; and was representative of the characteristics that influence growth such as birth weight, and parental height (WHO, 1995b). The new curves will serve as a standard to assess the growth of both breastfed and formula fed infants (Dewey 1998b). Representative curves will allow for adequate classification of stunting, wasting, malnourished, overweight, and obese (WHO, 1995a). The new curves are scheduled for release in 2006 (Frongillo, personal communication, August 2005). In the mean time, the experts suggest using the pooled data set of breastfed infants in conjunction with the WHO/NCHS curves when assessing for possible growth faltering (Dewey 1998b) or for international comparisons (Frongillo 2000b)

B. Determinants of infant growth

Although feeding is directly related to child growth, other factors influence growth through feeding practices. In 1990, UNICEF developed a conceptual model to represent the household and societal factors associated with infant health outcomes. Frongillo and Hanson (1995) adapted the model and presented three important causes of variability in child growth among nations: basic, underlying, and immediate causes (Figure 1). The basic causes include political, social, and economic structures and institutions. The underlying causes consist of food security, maternal and child care, health services, and physical environment. The underlying causes affect immediate causes such as adequacy of the diet and health status, which impact directly on growth. Growth studies have primarily focused on the relationship between immediate causes (adequacy of the diet and health status) and growth. Information generally collected at

baseline on maternal education, SES, age, and parity, tries to estimate the underlying causes that are responsible for influencing growth. In the paper presented by Frongillo and Hanson (1995), the analysis of country data showed that child weight and height were predicted by female education and access to safe water. Child height was also determined by the energy density of the diet. Importantly, height was found to be sensitive to the basic causes. That is, the more adverse the basic causes (political unrest, economic instability) the greater the impact underlying causes had on growth. Of the variability in weight and height among countries, 90% was accounted by previous growth; sex and ethnicity play a significant role as covariates in explaining length and weight. However, the authors cautioned not to confuse ethnicity with genetics because ethnicity may be a marker for underlying or basic causes such as feeding practices or economic stability (Frongillo and Hanson 1995).

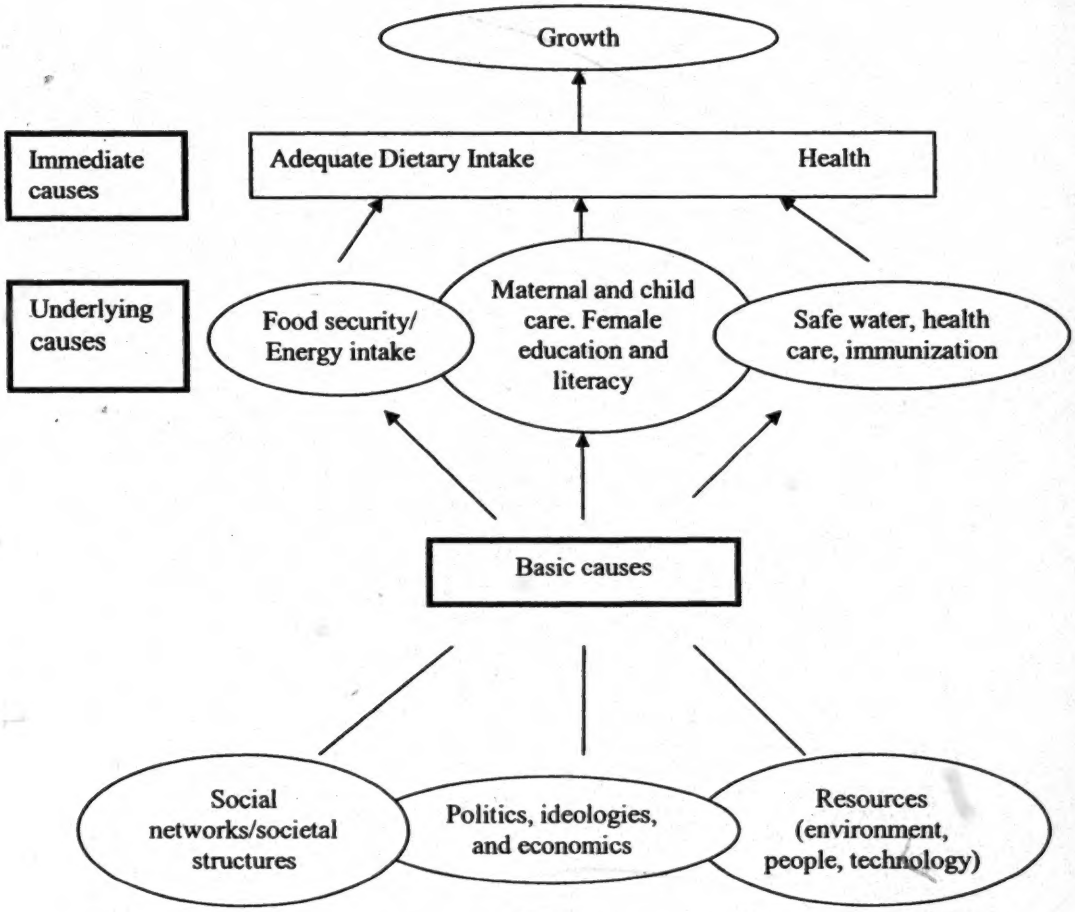


Figure 1. Factors associated with variability in child growth. Adapted from UNICEF, *State of the World's Children, 1998, Oxford University Press;* Frongillo and Hanson *Annals of Human Biology. 1995; 22: 395-411.*

Conversely, the theory of 'positive deviance' attempts to explain the adequate growth of children who live in adverse environments (Zeitlin 1991). This positive growth is linked to the success of 'maternal technology' and the appropriateness of " practices, traditions and beliefs relating to food preparation, feeding techniques, child care during illness and convalescence, handling of drinking water and feces, and personal hygiene" (p260). Positive deviance emphasizes that children who grow adequately do not live in completely opposite physical environments to those that are severely malnourished. Positive deviant children are those children who live in a cohesive environment, have

positive family dynamics and interactions, whose parents are less fatalistic and readily make use of health services and educational campaigns (Zeitlin 1991).

C. Growth and its relationship to feeding mode

(1) Studies in developed countries

Infants in developed countries, where the environment is conducive to maximum growth potential, are often regarded as the reference for healthy child development. One such US study that provided this type of information is the DARLING Study by Dewey and colleagues (1992). The researchers investigated the growth patterns, nutrient intake, morbidity of matched cohorts of breastfed (n=45) and formula fed (n=41) infants from 0-18 months (Dewey et al. 1992). BF infants were those who received breast milk for the first year of life, and no more than 120 ml/day of other milk or formula. Formula fed infants were those whose mothers chose to completely wean from breast milk by three months of age and given iron-fortified formula. At the end of the 18 month follow-up period weight was lower in both breastfed boys and girls as compared to FF, and BF infants attained a lower WLZ than FF infants. There were no differences in length at any time period between breastfed girls and formula fed girls; however in boys, cumulative length gain at 12 months was associated with formula feeding. The study also provided evidence that BF infants self-regulate their intake. Breastfed infants consumed significantly less milk and solid food compared to formula fed infants. Consequently, breastfed infants had significantly lower intakes of energy and protein at 3, 6, 9, and 12 months of age than those who consumed formula (Heining et al. 1993). The reduced intake did not adversely affect behavioural development or increase morbidity.

The DARLING study concluded that it is physiologically normal and healthy for BF infants to gain weight less rapidly than FF infants. In an optimal environment, a slower weight gain does not signify growth faltering, and breastfeeding should be continued for the first six months (Dewey et al. 1992). Salmenpera et al. (1985) also found reduced length velocity in breastfed infants during the 3-6, 6-9 and 9-12 month intervals. The researchers questioned if this type of growth was physiologically normal or if formula fed infants were overfeeding (Salmenpera et al.1985).

Another large study (PROBIT Study, Kramer et al. 2002) was undertaken in Belarus and involved 17 046 singleton, healthy term infants. The experimental group

was comprised of mothers who had given birth in a Baby Friendly Hospital and who after discharge from the hospital received community support to maintain exclusive breastfeeding; the control group had not given birth in a BFH and received standard care. Infants were followed at the polyclinics at 1, 2, 3, 6, 9, and 12 months. At each visit, details about infant feeding, gastrointestinal and respiratory tract infections, rashes, symptoms, and any hospitalization since the last visit to the polyclinic were collected, and anthropometry was measured (weight, length, and head circumference). Kramer et al. (2002) found that WAZ were above the reference mean for infants who were exclusively breastfed for three or six months. Similarly to the DARLING study, exclusively breastfed infants in the PROBIT study showed slower weight and length gains from 3-12 months and catch-up growth thereafter; but PROBIT breastfed infants remained above the reference mean in WAZ. This could be explained by biological and cultural factors specific to the population of Belarus (Kramer et al. 2002). At six months exclusively breastfed infants' LAZ fell below the reference mean but caught up to the mean at 12 months (Kramer et al. 2002). PROBIT infants show no signs of growth faltering with exclusive breastfeeding for up to six months (Kramer et al. 2002).

Kramer et al. (2003) compared the health and growth of 2863 exclusively breastfed infants from the PROBIT trial. The study found that WAZ and LAZ at six and nine months were slightly but significantly larger in infants who received complementary food after three months of age. At 12 months, WAZ was larger in infants EBF to three months; whereas, there were no significant differences in LA between the two groups. WL differences between the two groups were not significant at any age (Kramer et al. 2003). This is in contrast to the DARLING study where WL was lower in the breastfed children.

(2) Studies in developing countries

Cohen et al. (1994) undertook a randomized study to look at the effects of introducing complementary foods at four months versus six months with regards to breast milk energy intake and growth in Honduran infants. All 141 term infants were recruited at birth and had been exclusively breastfed until four months of age (16 wks). At 16 wks the infants were randomly assigned into three groups. One group continued exclusive breastfeeding until six months (26 wks); the second group introduced solid foods at 16

weeks and maintained the same breastfeeding frequency; and the third group introduced solid foods and continued breastfeeding on demand. The study found that there were no growth advantages to introducing complementary foods at four months of age. Breast milk intake reduced spontaneously when solid foods were introduced despite continual reinforcement of breastfeeding. The mean energy intakes of all three groups were similar during the intervention period. Similarly there were no significant differences in weight or length among the three groups at any time point. Although mothers had marginal diets, lactation performance and growth were not compromised; growth faltering was not observed. The researchers concluded that exclusive breastfeeding to six months and introduction of foods thereafter could be recommended in a low-income, high-risk (LBW infants and mothers with low BMI) population in a developing country (Cohen et al. 1994).

Cohen et al. 1995 also examined the determinants of growth in the Honduran cohort and found that there were no growth differences among the three feeding groups. Infant growth from birth to four months demonstrated a positive association with maternal height, sex of infant, and weight gain in the previous months, and breast milk intake (R^2=0.26). Fever was negatively associated with weight gain (-912 g) during 4-6 months, but illness was not (diarrhea and respiratory infections). Attained weight and length at 12 months were associated with birth weight, maternal height, and infant sex (R^2=0.31). The reduction in growth velocity after nine months might have resulted from the replacement of breast milk with less nutrient dense foods. The relationship between infant weight and length and maternal height indicates a genetic component but also a prenatal effect that persists post-natally (Cohen et al. 1995). Of particular interest, breast milk intake was significantly associated with growth from 0-4 months although the direction of causality was unclear (beta coefficient = 0.2). As in the DARLING study, in this cohort there was no association between illness and attained growth. The absence of an association may be related to the intrinsic factors of breast milk that reduce the incidence or severity of infections, which would normally impact growth (Cohen et al.1995).

Villalpando and Lopez-Alarcon (2000) followed the growth of 170 healthy term Mexican infants every two weeks at home or at the clinic to 6 months of age. Weight and

length was taken bimonthly and infants were classified according to the type of milk received in the first six months (BF, FF, or partially breastfed). At six months, BF infants were heavier with a tendency to be taller than FF infants. Weight at six months was associated with episodes of diarrhea and with the duration of breastfeeding (R^2=0.17). Thirty-two percent of FF infants were faltering (wasting, stunting or underweight) by 6 months in comparison with 8% of breastfed infants. The authors concluded that breastfeeding had a dose response relationship with growth by offsetting the effects of infection in infants who were ill. They speculated that infants who became ill and were breastfed were protected against illness induced anorexia (Villalpando and Lopez Alarcon 2000).

Eckhardt et al. (2001) followed 183 Mexican infants living in a semirural community to 20 months of age. At 20 months, infants that were fully breastfed (breast milk plus other liquids except infant formula or cow's milk) for at least four months were smaller (1.21 cm) and lighter (500 g) than infants not fully breastfed for at least four months. Interestingly, larger infants at 2-3 months were more likely to receive full breastfeeding; mothers who perceived having adequate lactation performance to support growth were less likely to wean from the breast (Eckhard et al. 2001).

Kramer and Kakuma (2001) undertook a meta-analysis of exclusive breastfeeding and health outcomes and found that, at the population level, the research suggests that there are no adverse effects on growth with exclusive breastfeeding to six months. The authors acknowledged that growth faltering in LBW infants or in BF infants whose lactating mothers are severely malnourished may be a concern. Nonetheless, the benefits of reduced morbidity and mortality may outweigh the growth risks, particularly in developing countries (Kramer and Kakuma 2001).

(3) Summary

Differences in growth between breastfed and formula fed infants Various well-designed studies have concluded that exclusively breastfed infants grow differently than formula fed infants even in environments that are conducive to reaching growth potential. Formula fed infants grow faster than BF infants, and BF infants grow differently than the growth reference curves would suggest (Garza et al. 1994). Dewey (1998 b, c) summarizes the growth patterns of BF infants and argues that the growth of BF infants be

considered optimal. The average trend of breastfed infants is to rapidly gain weight from birth to 2-3 months of age and subsequently gain less weight than the mean of the NCHS curves from 3-12 months. The downward trend is often interpreted as faltering. Studies carried out in Europe and North America have consistently demonstrated that breastfed infants deviate more in WA than in LA; thus at 12 months of age, WL is considerably lower than the mean. Disadvantaged populations show the same growth trend in weight and length of affluent populations; although, infants living in less protected environments may grow more quickly on breast milk than on formula (Dewey 1998 b, c).

Significance of growth differences between breastfed and formula fed infants The slower weight gain does not pose any functional problems (cognitive or motor development delays), and suggests that formula fed infants may gain excessive weight (Dewey 1998 b, c). Dewey et al. (1993) reported that BF infants (n=46), when compared to FF infants (n=41), had significantly lower body fat as evidence by smaller skin fold thicknesses (4-7 mm less than FF) from 9-15 months, and lower estimated percent body fat (2% less than FF) from 9-24 months. This evidence suggests that breastfed infants are leaner than formula fed infants. At 2 years of age, the 'growth deficiencies', exemplified by low WA and LA, are no longer apparent as anthropometric indices of BF infants are at the mean (Dewey 1998 b, c). On the other hand, Fomon (2004) argues that some breastfed infants are at risk of not reaching their growth potential because they fail to obtain a generous intake of nutrients present in commercial formula (energy, protein, and other essential nutrients). He argues that the accelerated growth of formula fed infants is indicative of better growth since it results in appreciable accretion of fat free mass (Fomon 2004). Thus, the current issues in breastfeeding practice center around the adequacy of energy intake to maximize or optimize infant growth in weight and length (Lutter 2000).

2.4. Infection and feeding mode

Infections may adversely affect the nutritional status of an infant because of their low reserves of fat, protein and glycogen, and higher basal metabolic rate and increased energy demands for growth (Fulhan 2003). Infections in the first six months of life may adversely affect growth through decreased appetite, decreased nutrient absorption,

increased nutritional requirements, or nutrient losses (WHO 1995b). The relationship between infection and nutrition is depicted in Figure 2. Malnourished children are characterized by a depressed immune system, and increased rates, severity, and risk of mortality from infections (Brown and Begin 1993).

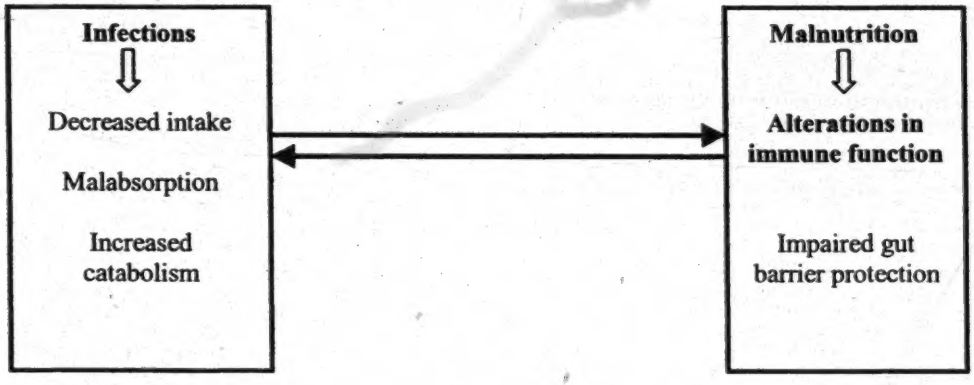


Figure 2. Relationship between malnutrition and infection. Adapted from Brown, *J Nutr. 2003; 133: 328S-332S.*

Micronutrients play an important role in modulating the immune system and reducing the risk of infections. For example, Vitamin A deficiency is a risk factor for persistent diarrhea (Shahid et al. 1988). In India, vitamin A supplementation (60 mg/day) in iron deficient children (1-5 year olds, n=451) was shown to reduce the severity and risk of persistent diarrhea (OR=0.30) (Bhandari et al. 1997). Zinc deficiency has also been linked to longer duration and severity of diarrheal symptoms (Sazawal et al.1995). A study in India that provided a daily zinc supplement (20mg of elemental zinc) to children ages 6-35 months (n=456) found a 23% reduction in the risk of continued diarrhea, a 39% decrease in the number of watery stools per day, and a 21% reduction in days ill with diarrhea (Sazawal et al. 1995).

Because an infant's immune system develops during early infancy (Field 2005), breastfeeding reduces the burden of disease and may guard against mortality by providing protection against gut enterotoxins (Morrow et al. 2004). For example, antibodies present in breast milk, which provide immune protection, are specific to the mother's infectious endemic environment (Morrow et al. 2004). Also, breastfeeding reduces the anorexic

response (Lopez-Alarcon et al. 1997) and the impact of gastrointestinal illness through immune components (Brown 2003). Diarrhea, as a direct result of an enteric infection, has the most profound nutritional impact (WHO 1995b). Diarrhea is a mechanism by which the body rids itself of microorganisms (Long et al. 1999). The maximum protection against diarrhea is conferred by EBF (Morrow et al. 2004).

Factors associated with infectious diarrhea, derived from cross-sectional studies, include wasting and underweight. Other risk factors for diarrhea are infant feeding practices (lack of exclusive breastfeeding, contaminated weaning foods and liquids), micronutrient status (vitamin A or zinc deficiency) (Brown 2003), a mother's level of education (more education is important in ending infections sooner) (Long et al. 1999), and income (poor socioeconomic status) (Macias-Carillo et al. 2005). Acute diarrhea is associated with crowding, environmental contamination, lack of potable water, and lack of medical attention (Macias-Carillo et al. 2005).

A. Immunological benefits of breast milk

Bioactive factors in breast milk act as ligands for bacteria and viruses, and also aid in the maturation of the infant's immune system via maturation signals (Hamosh 2001). Breast milk contains antibodies or immunoglobulins A (IgA), G (IgG), and M (IgM). IgA comprises 90% of the immunoglobulins (Newburg 2005) and it prevents adherence of pathogens in the gut epithelium (Morrow et al. 2004). Proteins present in milk also provide immunological benefits. For example, casein, the main protein in breast milk, acts as an anti-adhesive for bacteria (Hamosh 2001). Lactoferrin, the transport protein for iron, promotes intestinal growth, stimulates growth of probiotic bacteria in the gut, acts as an antiviral, is an antiadhesive for *Escherichia coli* (Hamosh 2001), and modulates cytokine production (Morrow et al. 2004).

Oligosaccharides and glycoconjugates, such as mucin-1 and lactadherin, prevent the binding of microorganisms and their toxins to epithelial cells (Hamosh 2001). They also inhibit rotavirus infection *in vivo,* and *in vitro* have been shown to inhibit binding of *Vibrio cholerae, E. Coli, Haemophilus influenzae,* and *Streptococcus pneumoniae* (Hamosh 2001). Glycans predominantly inhibit the pathogen's capacity to infect the host but also promote growth of beneficial bacteria in the colon (Morrow et al. 2004).

Specifically, lactose and oligosacharrides are probiotic factors promoting the formation of bifidobacteria, which are necessary to support the gut barrier function (Morrow et al. 2004). Probiotic bacteria provide maturation signals via Gut Associated Lymphoid Tissues and regulate the local inflammatory response (Morrow et al. 2004).

Fatty acids such as lauric and linoleic acid, act as ligands, and promote the lysis of bacteria. Products of triglyceride digestion, monoglycerides, are antiprotozoan (*Giardia lamblia*), antimicrobial *(H. influenzae)*, and antiviral (herpes simplex virus) (Hamosh 2001).

Breast milk has many anti-inflammatory agents such as Vitamins A, C, and E, catalase and glutathione peroxidase, IL-10, anti-inflammatory receptors for TNF α and IL-1, all of which reduce the anti-inflammatory response in breastfed infants both in the gut and systemically (Hamosh 2001). The presence of nucleotides and cytokines in breast milk enhance an infant's immune response by promoting cell mediated immunity (leukocytes, macrophages, and natural killer cells) (Field 2005; Morrow et al. 2004). Glycosylation and phosphorylation of milk components in the mammary gland allows the immune components to remain bioactive in the gut (Hamosh 2001). Immune cells such as leukocytes may possibly migrate into the circulation (Hamosh 2001).

B. Research on infant infection and feeding mode

A study in Guadalajara investigated the relationship between heat labile toxin producing *E. Coli* infection and feeding practices in the first year of life. Healthy term infants (n=98) were visited 2-3 times per week until their 12th month birthday. Stool and breast milk samples were collected weekly. Diarrhea was defined as $\geq$4 liquid or semi-liquid stools per day. Long et al. (1999) found that after colonization with *E. coli* breast milk reduced the likelihood of becoming symptomatic in a dose dependent fashion (at least 3 breast feedings/day) but did not decrease the duration (days) of symptoms. They specified that being non-symptomatic despite colonization was attributable to passive immunity received through breast milk. The use of teas and rice water decreased symptoms but increased the time the infant remained infected, although, asymptomatic. Previous infections from *E. coli* were more likely to increase immunity to the pathogen (active immunity) and decrease the duration of symptoms. The authors concluded that infants in developing countries were more likely to have asymptomatic infections versus

symptomatic infections; passive (through breast milk) and active immunity were more related to the prevention of disease onset (symptoms) versus the actual disease infection (Long et al. 1999)

Macias-Carillo et al. (2005) followed 329 infants in Durango, Mexico from birth to 3 months with bimonthly home visits. Information was collected on acute diarrheal illness and feeding practices. From 0-3 months, infants were classified as EBF (48.1%), partially breastfed (32.0%) and formula fed (20.0%). Overall, 28% of the infants had at least one case of diarrheal illness, and 10% of those cases occurred in the first month of life. Those at risk were PBF (RR=3.2) and exclusively FF (RR=4.3) infants (Macias-Carillo et al. 2005). Unfortunately, the variable diarrhea was not specifically defined (duration, type, and number of symptoms per day) in this study.

Because of the numerous immunological components of breast milk, theoretically, a dose response relationship should exist between breastfeeding and infections. In addition to the passive immunity provided in breast milk, there exists the physical barrier of the breast that prevents contamination with enteric pathogens (Lopez-Alarcon et al. 1997). Kramer and Kakuma's (2002) meta-analysis concluded that exclusive breastfeeding was protective against gastrointestinal infections up until six months of age in all types of living conditions. Less clear is the effect of breastfeeding on upper respiratory tract infections. In respiratory infections there is no physical barrier by offering the breast. Any protection extended by breast milk depends on its antimicrobial or immunomodulating properties (Lopez-Alarcon et al.1997) afforded through passive immunity.

In Denmark, Rubin et al. (1990) developed a prospective observational study to describe the relationship between infant feeding and infections. The researchers followed 500 term infants with a birth weight of >2000g. Mothers completed a monthly questionnaire which asked detailed information about the type and duration of their infant's symptoms, how the mother dealt with those symptoms (called a doctor or a friend), any illness in the household, feeding frequency, solid foods given to infant, and child care arrangements. Feeding frequency was used to define five breastfeeding categories. The classification of feeding was done independently of solid food intake. Gastrointestinal illness was defined as having at least 2 symptoms with duration of 2-20

days: a fever of 38.5 ℃, increased stool frequency, loose stools, or vomiting. Respiratory tract infections were defined as having at least 2 symptoms with duration of 2-20 days: a fever of 38.5 ℃, a runny nose, fast breathing, cough, or diagnosis by physician. The authors found that monthly incidence of infections were equal between FF and BF infants. After controlling for significant confounders, the authors concluded that there was no substantial protective effect of breastfeeding against gastrointestinal infections or respiratory infections, in middle class urban population in Denmark (Rubin et al. 1990).

Dewey and associates (1995) in the DARLING study compared the morbidity rates of breastfed infants (n=45) to formula fed infants (n=41). Mothers were given a daily calendar to record their infant's symptoms and were contacted weekly about the nature and duration of symptoms and if any diagnoses had been made by a physician. Respiratory illness was defined as any upper or lower respiratory tract symptom other than clear nasal discharge. Diarrhea was defined as two or more runny stools a day. After controlling for confounders there was no difference in URTI between FF and BF infants. The incidence of diarrhea was significantly higher, twice as much, in FF infants than BF infants. Dewey and colleagues concluded: "the reduction in morbidity associated with breastfeeding is of sufficient magnitude to be of public health significance" (p 696).

A study by Lopez-Alarcon et al. (1997) studied healthy term infants (n=170), who weighed more than 2500g, were recruited at birth and followed every two weeks to six months. Infants were classified as fully breastfed or FF if they had not been fed any other type of milk in the previous month. No mention of the use of solid food or other liquids was specified in this study. Mothers kept a daily record of their infant's illness and symptoms. Diarrhea was defined as having more than three liquid stools per day, with or without a fever. Acute respiratory infections were defined as a cough or runny nose for more than two days with one or more of the following symptoms: erythematous mucosa, hoarse cry, respiratory distress, and fever. The authors found that 25% of infants were exclusively breastfed to 6 months. During the first four months of life FF infants had increased risk ($1.86>RR<5.56$) of suffering an episode of respiratory but after four months the difference became non-significant. Infants with at least one sibling who were never breastfed were more likely to have an episode of acute respiratory infection that lasted longer (6.4 days) than those who were fully breastfed (5.1 days) for at least one

month. Also, the duration of diarrhea was longer for FF infants (6.2 days) than for infants fully breastfed (3.8 days) (Lopez-Alarcon et al.1997)

The PROBIT study was designed to assess the benefits of exclusive breast milk and infectious outcomes. The primary outcome of the study was one or more episodes of gastrointestinal illness. Secondary outcomes were at least two episodes of respiratory tract infection, which included URTI, otitis media, croup, wheezing or pneumonia. Gastrointestinal illness was defined as having at least two symptoms with a minimum duration of two days: fever of 38.5 ℃, increased stool frequency, loose stools, or vomiting. Respiratory tract infections were defined as having at least two symptoms with a minimum duration of two days: fever of 38.5 ℃, a runny nose, fast breathing, or cough. The intervention group (EBF to six months) had significant risk reduction for gastrointestinal infections (OR=0.60); however he protection did not extend beyond the period of exclusive breastfeeding. There was no significant risk reduction in URTI in the intervention group. The authors question if this was due to the high initiation and breastfeeding rates in both the control and intervention groups or if breastfeeding does not have a protective effect against respiratory tract infections (Kramer et al. 2001).

Raiser et al. (1999) analyzed the data from the US 1988 National Maternal and Infant Health Survey to assess for a possible dose response relationship between infant feeding and illness. Mothers (n=5000) recorded the number of episodes and symptoms of illness in the first six months of life, feeding frequency in one of five categories (exclusive breast; breast>other, breast=other, other>breast, no breastfeeding), type of milk (breast milk or formula) and type of solid food the infant received during the first six months of life. After controlling for birth weight and other confounders, the authors found that exclusive breastfeeding was associated with reduced likelihood for diarrhea (OR=0.54), and vomiting (OR=0.71). When compared to EBF, no breastfeeding had significantly higher odds ratios for diarrhea and vomiting, 0.95 and 1.11 respectively. Breastfed infants without siblings had lower odds ratio for ear infections (OR=0.49), colds (OR=0.69), and fevers (OR=0.71). Exclusively breastfed infants had less sick medical visits. The authors concluded that a dose response with breastfeeding was seen for both diarrhea and colds in infants without siblings only. Minimal breastfeeding did not have any reduced risk for any infectious illness. One major drawback of this study

was that it relied on mailed questionnaires and it was subject to a variety of errors including maternal recall, and inability to verify medical information provided (Raiser et al 1999).

2.5. Iron status

Iron is indispensable in various metabolic processes. Specifically, iron functions as an oxygen carrier via hemoglobin, as a red-ox agent in the electron transport chain, and as a co-enzyme in many tissues (FAO 2001). Iron is necessary for the functioning of muscles (myoglobin) and the brain (FAO 2001). Depletion of tissue iron could impair adequate functioning of these processes. In animal studies the causal link between ID in the postnatal period and adverse neurological and behavioural outcomes has been established (Beard et al. 2002; Golub et al. 2005). However, a causal link between these metabolic processes and iron deficiency has been difficult to prove in infant research studies.

A. Consequences of iron deficiency and anemia in infants

Iron deficiency The immune system requires sufficient amounts of iron for adequate DNA synthesis in T-lymphocytes, for neutrophils to phagocytose, and for respiratory burst of immune cells. ID may adversely affect the functioning of the immune system making infants susceptible to infections (FAO 2001). Iron deficiency may reduce infant appetite (Groff and Gropper 2000). The brain uses iron in myelination, and formation of neurotransmitters; thus, iron depletion may adversely affect behaviour, learning, and motor functioning (Oski 1993).

Iron deficiency is defined by the deletion of iron stores. Serum ferritin <12 μg/L (WHO 2001a) or <10 μg/L (Wu et al. 2002) is a measure of iron deficiency in infants. A ferritin value <12 μg/L is usually interpreted to mean the exhaustion of iron stores (liver, spleen, and bone marrow; Groff and Gropper 2000) thereby compromising supply of iron to the tissues (FAO 2001), which may impair the functioning of muscle, tissues, brain, and erythrocyte (Stoltzfus 2001). In practice IDA is a functional concept for ID. Anemia per se is important only when oxygen delivery becomes compromised (FAO 2001). Thus, the focus has shifted from preventing IDA to preventing ID (Fomon et al. 2000).

Establishing a definite causal link between ID and developmental effects has been difficult, in part, because psychometric tools are unable to truly measure changes (Pollit 2001). Development is complex. Many of the factors involved in shaping infant development are not static (Pollit 2001). In his review Pollit (2001) explains that development is determined by highly influential psychobiological factors such as social context (culture, community, education of parents, and education of the child), biology (neuronal development, health, and nutrition), and individual experiences (life events and the microenvironment of the infant). A plausible biological hypothesis, as demonstrated in animal models, suggests that ID initiates a cascade of cerebral changes that result in a decreased mental capacity. It is also possible that IDA, through decreased oxygen supply to the brain and muscle, negatively impacts motor development and reduces physical activity in infants. Yet, these relationships remain to be established in infants and toddlers (Pollit 2001).

Anemia In infants, anemia is clinically defined as a low concentration of hemoglobin in the circulating erythrocyte and is diagnosed by Hb <110 g/L (FAO 2001). Hemoglobin synthesis occurs in the erythropoietic cells of the bone marrow (Groff and Gropper 2000). Infants with adequate iron stores normally have hemoglobin levels greater than 110 g/L (Fomon et al. 2000). Changes in hemoglobin concentrations and erythrocyte morphology occur after iron stores are exhausted (Fomon et al. 2000). In theory, iron deficiency precedes anemia if the cause of the anemia is depleted iron stores; however, this sequence is not always observed in early infancy (Domellof et al. 2002). In infants, when iron stores are limited, iron is preferentially shunted to erythropoiesis leaving the brain, heart and muscle at risk (Lichtman et al. 2006). Thus, diagnosis of severe anemia using Hb remains straightforward, but it is more difficult to interpret mild anemia or a normal Hb concentration coupled with ID; both which could have potential serious public health implications (Stoltzfus 2001).

Lozoff and colleagues (2003) recruited 1657 healthy 6-month old Chilean infants to evaluate the effects of iron supplementation on behavioural and developmental outcomes. At study entry infants were free of iron deficiency (<12μg/L) and were randomized to receive iron or no iron to 12 months of age regardless of their feeding mode. Although there were developmental differences between the groups, those

differences were small in magnitude: At 12 months, infants who were not supplemented with iron crawled a week later, had poorer social and emotional function as assessed by the Fagan Test, and were less efficient at processing information (predictive of cognitive function later on in life) than those who were supplemented. There were no differences between groups in motor and mental test scores as assessed by Bayley's Scales of Infant Development. Iron deficiency anemia (Hb <110g/L and ferritin <12μg/L) was low in the supplemented group (3%) yet 26.5% were iron deficient despite supplementation. In the unsupplemented group, IDA and ID at 12 months were 22.6% and 51.3%, respectively (Lozoff et al. 2003).

Dewey's research group (2001), using the data (n=260) from two Honduran trials, assessed the effects of EBF to 4 months versus 6 months on functional outcomes. Despite having low iron stores at 6 months of age, infants EBF to six months crawled earlier and were more likely to walk by 12 months. Although the results of this study were based on maternal report of motor developmental milestones, the authors argued that the amount of breast milk provided to the infant from 4-6 months may be enough to play a role in brain and motor development (Dewey et al. 2001).

B. Iron metabolism in infancy

In a newborn, both serum ferritin concentration and erythrocyte ferritin are much higher than that of an adult (Blot et al. 1999). At birth an infant has approximately 75mg/kg of body weight of iron, which translates into about 200-300 mg of iron. *In utero*, iron absorbed by the fetal erythroblast exceeds what is needed for hemoglobin synthesis; the excess iron is stored as ferritin in the erythrocyte (Blot et al. 1999). Consequently, the high erythrocyte ferritin concentration serves as a reservoir of available iron in the months following birth (Blot 1999). The amount of oxygen available to the newborn infant is more than what was available *in utero,* causing a decrease in hemoglobin production from birth until 2 months of age and a gradual decrease in hemoglobin concentration (FAO 2001). This phenomenon is known as physiological anemia. The iron released from the natural breakdown of senescent erythrocytes in the first two months are shunted into storage, which will cover the iron needs of an infant to about 4 months of age (FAO 2001). After 2 months of age, hemoglobin levels stabilize and iron levels begin to fall slowly as iron is utilized for Hb synthesis (Ulukol et al.

2004). A fall in hemoglobin concentration from 2-6 months is probably a consequence of iron deficiency (Ulukol et al. 2004).

C. Requirements

Daily minimal requirements for bioavailable iron in infants are estimated at 0.3 mg/day for the first year of life (Bothwell et al. 1989). Adequate Intake of 0.55-0.75 mg/day from 0-6 months is recommended (Institute of Medicine (IOM) 2001). Others recommend 0.7-0.9 mg/day from 4-6 months of age (FAO 2001). Breast milk iron concentration ranges from 0.5 mg/L in the first month to 0.35 mg/L at six months (Butte et al. 2002). About 50% of iron in breast milk and 7% of iron in iron fortified formulas (12mg of iron/L) is absorbed (Fomon 1993; AAP 1999).

D. Iron retention and absorption in infancy

Fomon et al. (2000) discusses the myriad of factors that modulated iron retention in infancy. Iron retention increases with age, low birth weight, and presence of iron deficiency. Conversely, iron retention decreases with increasing iron dose. Not all of the iron that is administered orally is absorbed. Some remains in the enterocyte without ever being released systemically and is eventually sloughed off and lost in the feces. In infants, only about 50% of iron retained is incorporated into erythrocytes, which differs from adults who incorporate 80-100% of iron into erythrocytes (Fomon et al. 2000).

Iron retention is also influenced by the type of food an infant consumes. For example, supplemental iron is better retained when given in absence of food, and is much better absorbed than heme iron present in a meal. Cow's milk, formula, or food given before or after a supplemental dose influence iron retention. Human milk has a low content of non-heme iron inhibitors and BF infants retain a low dose of supplemental iron better than FF infants (Fomon et al. 2000). However, more research is needed to understand iron retention under usual infant feeding conditions in order to maximize utility of dietary and supplemental iron (Fomon et al. 2000).

Sources of heme iron in a weaning diet include meat, poultry, fish and egg yolks; only 25% of the heme iron is absorbed (FAO 2001). Sources of non-heme iron include

cereals, pulses, legumes, and fruits and vegetables; only 10% of non-heme iron is absorbed (FAO 2001). The absorption of non-heme iron in a meal is further modified by the presence of inhibitors or enhancers of iron absorption. Known inhibitors of non-heme iron include phytates, calcium (also an inhibitor of heme iron), and phenolics found in green leafy vegetables. At least 25 mg of vitamin C converts non-heme iron to its reduced form facilitating absorption in the gut. Protein, such as meat, fish, and seafood, also enhance absorption of non-heme iron but the gut process remain unknown (FAO 2001).

E. Factors that influence iron nutriture in infancy

There are three main factors that act in concert to influence iron status at six months of age: maternal factors, biological factors, and feeding practices.

(1) Maternal factors

Undoubtedly, the principal endowment of iron occurs *in utero*, specifically in the third trimester of gestation (Blot et al. 1999). Any abundance or deficits in maternal iron status may increase or limit the amount of iron a fetus can accumulate (Blot et al. 1999).

In pregnancy, a low hemoglobin concentration is used as a proxy for iron deficiency. Because of hemodilution (expansion of blood volume), hemoglobin is normally depressed (<110g/L) in pregnancy and the current cut-off for Hb may not be indicative of true iron deficiency (Rasmussen 2001). Currently, hemoglobin concentrations of 100-110 g/L are used to define nutritional anemia (iron, folate or B_{12}) (Blot et al. 1999), but maternal Hb concentrations between 100-110 g/L may not impair iron accretion in the fetus (Rao and Georgieff 2002). Even though the biological pathway is likely, establishing a causal relationship between mother's iron status and neonatal status at birth has been a challenge. The literature has shown that iron status at birth does not differ between anemic (Hb: 100-110g/L) or non-anemic mothers (Blot et al. 1999 and Vasquez-Molina et al. 2001). Still, the effects of maternal supplementation on infant iron stores may not become apparent until the infant is 3 or 6 months old (Rasmussen 2001). Maternal supplementation positively affects newborn iron status if the mother is iron deficient as measured by serum ferritin < 120 μg/L (Blot et al. 1999) or Hb of <80g/L (Rasmussen 2001).

Other factors linked to anemia in infancy are malaria, and hookworm (WHO 2001b).

(3) Feeding practices and iron status

In most affluent populations iron deficiency prevalence is low because of the use of iron-fortified infant formula (Fomon 2001), iron-fortified cereals (FAO 2001), or both. Some pediatricians recommend prophylactic iron drops shortly after birth to prevent depletion of iron stores in predominantly breastfed infants (Fomon 2001). Although the concentration of iron in breast milk is low the bioavailability is high, and thus not all EBF infants become iron deficient (Butte et al. 2002). In some affluent populations, iron deficiency among full-term, EBF infants is rare at six months of age (Dewey et al. 1998). Conversely, in populations where maternal iron stores are poor and infant's stores are not optimal, iron status of EBF infants at six months is low compared to partially breastfed infants at four months of age (Kramer and Kakuma 2001). Therefore, exclusive breastfeeding until six months of age may contribute to iron deficiency in susceptible infants (Kramer and Kakuma 2001).

Thirty healthy term Italian infants were followed from 6-24 months (Pisacane et al. 1995). Groups (EBF < 7 months vs. ≥7 months) were comparable in SES, birth weight, mean weight gain from birth, and daily consumption of heme containing foods. The EBF infants were less likely to be iron deficient (serum ferritin <10 μg/L) at 12 months (22%) than infants EBF for <7 months (52%) ($p=0.003$). Infants EBF ≥ 7 months were all free from anemia (Hb <110 g/L) at 12 months (Pisacane et al. 1995). On the other hand, Hadler et al. (2004) in their cross-sectional study of Brazilian infants found that those at risk for anemia (Hb <110 g/L) at nine months were exclusively breastfed for more than six months.

A study of Chilean infants (n=854) evaluated iron status at nine months based on method of feeding from birth (Pizarro et al. 1991). The cohort consisted of low SES infants who received regular medical care, lived in a clean environment (running water, indoor toilets, and electricity), had low rates of illness, and normal growth. Of infants who received solely human milk from birth to nine months, 35% had low ferritin (<10 μg/L) versus 12.3% of infants partially breastfed to six months and formula fed thereafter. The authors concluded that a good supply of iron is needed after six months of

age either as iron fortified formula or iron supplements to prevent ID (Pizarro et al. 1991).

In order to prevent ID in EBF infants two strategies are often prescribed: one, that foods be introduced at around 6 months of age; and two, that prophylactic iron drops (1g/kg/day) be administered. One study in Honduran infants evaluated the effects of introducing iron rich foods at 4 months and its usefulness in preventing ID at six months. Infants who received iron fortified foods from 4-6 months had significantly lower risk of ID at six months (7%) (ferritin <12 μg/L) when compared to EBF from 0-6 months (16%.) Infants receiving complementary foods were less likely to have low Hb (55%) when compared to the EBF group (66%) (Dewey et al. 1998); yet the prevalence at six months is quite high. Iron fortified foods from 4-6 months were insufficient to normalize hemoglobin and ferritin in all infants.

In a similar study, Honduran infants (n=131) who were supplemented with prophylactic dose of iron from 4-6 months had reduced linear growth if Hb concentration at four months was =110 g/L (Dewey et al. 2002). In infants with a Hb <110g/L at four months, iron supplementation from 4-9 months did not alter growth but did reduce the incidence of diarrhea; whereas, infants with adequate baseline Hb had increased incidence of diarrhea. The authors concluded that prophylactic iron supplementation 1mg/kg/day from 6-9 months of age in infants where IDA is prevalent would be warranted but caution against using supplementation in iron-replete populations secondary to the observed adverse affects on growth and health in infants with adequate Hb levels (Dewey et al. 2002). In infants less than six months of age, serum ferritin concentration, iron absorption, hemoglobin synthesis, and erythropoeisis may be regulated independently of each other (Domellof et al. 2001). Because of this immature homeostatic control, iron supplementation in infants who have adequate iron stores may have adverse effects (Dewey et al. 2002).

2.6 Assessment and Methodologies

This section provides a discussion on the assessment and methodologies related to each section of the literature review: infant feeding, growth, infection, and iron status.

Topics chosen serve as the scientific reasoning for the methods utilized in data collection and statistical analysis.

A. Infant feeding

To facilitate comparisons among studies on the effects of infant feeding and health outcomes, it is important that breastfeeding be defined consistently and clearly. Labbok and Krasovec (1990) underscored the necessity to describe exclusive breastfeeding as solely the use of breast milk, and almost exclusive breastfeeding when breast milk is given with vitamins, minerals, nutritive (juices, rice water) and non-nutritive liquids (teas, water). Partial breastfeeding should be defined as breast milk in combination with solid food, infant formula, and/ or cow's milk. The partial breastfeeding group can be further subdivided into high, medium, or low with regard to the amount of breast milk received. Subdividing the partial breastfeeding category permits assessment of dose response relationships in illness or nutritional status. Token breastfeeding (breast milk given occasionally or irregularly) should also be included as to not obscure or attenuate the relationship between breastfeeding and infant health. In 1991, the WHO adapted these definitions and decided on the following categories: Exclusive breastfeeding as no other liquids or fluids (except vitamins or medicines) including water; predominant breastfeeding as breast milk and the use of nutritive and non-nutritive liquids (no human milk substitutes or solid foods); and complementary feeding as breast milk in combination with solid food, infant formula, or cow's milk. (WHO 1991)

The WHO recommends using a 24-hour recall period to assess current feeding practices. This method has been widely used and found appropriate in surveys of dietary intake (WHO 1991). For completeness, caregivers should be asked frequency, duration, time intervals between feedings, and if other liquids or solids have been given to the infant (Labbok and Krasovec 1990; WHO 1991).

B. Infant growth

Pioneering studies in the area of infant nutrition suggested that breast milk had a strong beneficial effect on infant health (Bauchner et al. 1986). However, these studies have been challenged due to errors such as reverse causality and detection bias (Bauchner

et al. 1986). Reverse causality is when the outcome variable is a causal factor of the predictor variable (Marquis et al. 1997). For example, the non-initiation of breastfeeding due to an early death or switching feeding mode due to poor health of the infant (Villalpando and Hamosh 1998); thus reverse causality causes an overestimation of the beneficial effects of breastfeeding (Habicht and colleagues 1986). Bauchner et al. (1986) emphasize that detection bias is a common error in most prospective studies. Detection bias refers to one outcome event being detected more readily in one group than in the other. Active surveillance of participants can eliminate detection bias by ensuring that outcomes are equally detected in all groups. Also, failure to control confounding variables such as socioeconomic status, smoking, education, size of family, and/or vague definition of the outcome event have resulted in mixed conclusions regarding the benefits of breastfeeding (Bauchner et al. 1986). Other issues affecting conclusions are the type of statistical analysis, and the use of procedures that do not adequately measure the outcome of interest (Habicht et al. 1986). The prospective studies presented previously in this literature review eliminated these sources of error.

(1) Anthropometry

Anthropometry is indispensable for the growth monitoring of infants. Anthropometric measurements can be converted into Z-scores, which allow for comparison across groups of infants (WHO 1995a). A z-score is obtained by subtracting an infant's measurement from the reference mean and dividing the difference by the standard deviation of the reference population. Z-curves have a mean of 0 and a standard deviation (SD) of 1. Z-curves unit of measurement are standard deviation units.

An anthropometric measurement should be reliable and valid. Reliability refers to the degree to which a measurement is reproducible over time (Last 2001). Reliability can be affected both by imprecision (variability of measurements among the measurers and within each measurer) and by undependability (variability of the measurement due to physiological variations) (Ulijaszek and Kerr 1999). The reliability (between and within measurers) of a measurement can be increased with adequate training, standardized measurement technique, reducing the number of measurers, and periodic quality control throughout the study (Ulijaszek and Kerr 1999; Ferrario et al. 1995). Validity is how well the measurement actually measures a characteristic (Last 2001). Validity can be increased

by reducing inaccuracy. Inaccuracy is defined as a systematic bias due to instrument error or measurement technique (Ulijaszek and Kerr 1999).

a) Weight: a measure of total body size (Lohman et al. 1988). Weight is often measured with sufficient accuracy (Lohman et al. 1988). However, weight may be subject to error when an infant is restless, in which case the measurement should be retaken later. Accuracy can also be improved if there is adherence to a standardized technique (Lohman et al. 1988). Measurement error associated with infant weight is 240 g (WHO 1995b).

b) Length: measures total skeletal length (Lohman et al. 1988). For length to be a reliable and valid measure, strict adherence to the measurement technique is required. For example, sources of error include head not touching the headboard, legs that are not completely extended, or applying too much pressure on the soles of the infant's feet with the sliding foot board. Reliability and validity in length may also be affected by the cooperativeness of the infant (Lohman et al. 1988). Training measurers, using standardized techniques, and taking measurements on cooperative infants all help increase the validity and reliability of length measurements (Zerfas 1979). Measurement error associated with infant length is approximately and 0.6 cm (WHO 1995b).

Despite these technical issues, if measurers are trained in anthropometry, weight and length are simple, reproducible, and valid measurements to evaluate growth and the adequacy of the diet on growth (WHO 1995a).

(2) Anthropometric indices

This section summarizes the discussion found in 'The Use and Interpretation of Anthropometry' (WHO 1995a), unless otherwise specified. The focus is on factors affecting growth in developing countries.

(a) Birth weight: According to the WHO (1995a), birth weight is one of the most important indicators in assessing the duration of gestation and the average rate of intrauterine growth. LBW infants are those born at term ($\geq$ 37 weeks of gestation) but have a birth weight below 2500 g. LBW is used as a proxy for IUGR (WHO 1995a). Fetal growth is determined by maternal height, pre-pregnancy weight, energy intake during gestation, smoking, length of gestation, and genetics or racial background. During pregnancy, infections, hypertension, and pre-eclampsia may also influence fetal growth.

Impairment of fetal growth leads to increased risk of mortality and morbidity, and reduced growth and performance in the post-natal life. Birth weight should be determined within 12 hours of birth.

(b) Length for age: an index of linear growth. A low LA is termed shortness, if it is biological, or stunting, if it is a pathological process. Pathologically, a low index may signal long-term, cumulative inadequacies in health or nutrition. Using Z curves, stunting is defined as 2 SD below the mean.

(c) Weight for length: an index of weight relative to length. Field workers use WL because the age of the infant does not need to be known in order to interpret the index. A low WL indicates thinness or wasting. The term thinness refers to a biological process, whereas wasting refers to pathological processes that result from acute starvation or disease. Low WL can also signal chronic inadequacies of diet, or a chronic disease. Using Z curves, wasting is defined as 2 SD below the mean.

(d) Weight for age: an index of body mass relative to age. It is influenced both by the length and weight of an infant, thus is difficult to interpret. A low WA index refers to lightness (biological) or underweight (pathological). This index may express long-term inadequacies in health. In the short-term, a low WA will also cause a low WL. Using Z curves, underweight is defined as 2 SD below the mean.

(e) Head circumference for age: not used to assess nutritional status directly but it is an important indicator for assessing growth (brain size) in the first three years of life (Mexican Academy of Pediatrics 1996; Lohman et al. 1988).

Normally, stunting is more prevalent than wasting. Stunting may start as early as 3 months. Underweight among young children is more likely to reflect wasting but as children grow older underweight reflects stunting (WHO 1995a).

C. Measuring infectious morbidity

One of the issues researchers find most difficult is standardizing the measurement of infection. Of the prospective studies included in this literature review, various methods were used for follow-up, recording of symptoms, and diagnostic criteria. Follow-ups were necessary to prevent biases such as detection bias, maternal recall bias, overreporting and underreporting bias. However, not all studies had the same follow-up periods. For example, some researchers (Dewey et al.1995, Long et al. 1999) followed

dyads on a weekly basis and enquired about the type and duration of the infant's symptoms. Rubin et al. (1990) sent out monthly questionnaires and asked specific questions regarding symptoms, duration, or medical visits while ill. Lopez-Alarcon et al. (1997) followed infants every two weeks and whenever they were ill. The PROBIT study followed infants at the routine well-baby visits and whenever they were ill. In this study, pediatricians enquired about symptoms and hospitalizations since the previous visit (Kramer 2001). To help reduce maternal recall, Dewey et al. (1995) and Lopez-Alarcon et al. (1997) provided mothers with a daily calendar to record their infant's illness. Some studies even checked medical records for symptoms or diagnoses (Kramer et al. 2001; Lopez-Alarcon et al. 1997). Consistent definitions of illnesses are also important when comparing studies. Diagnostic criteria and the definition of an episode differed among the studies with the exception of Kramer et al. (2001) and Rubin et al. (1990), even though all studies had clear definitions of GI and URTI. The inconsistency in symptom definitions may add to the conflicting results among these studies.

Ideally, infants should be followed in a timely consistent manner; follow-ups should be frequent enough to prevent maternal recall bias (monthly, bi-monthly, or weekly follow-ups). Unfortunately, follow-ups are costly; they require time from the participants, and sufficient personnel. When more frequent follow-ups are not feasible, tools should be made available to the mother to record the infant's symptoms as they occur. However, in some instances, the mother may forget to record the symptoms and will provide the information based on recall. If the infant was treated by another physician, the medical record should be checked for possible diagnoses related to the study outcomes. Yet, accessing medical records from other physicians may not always be practical and relying on maternal recall of the diagnosis may be more realistic.

D. Iron status

(1) Indicators

Cook and Skikne (1989) provide a comprehensive review on iron status assessment and their paper is used in this section, unless otherwise specified. Firstly, it is important to differentiate between functional and storage iron. Functional iron exists mainly as hemoglobin in the erythrocytes and different tissue enzymes. Functional iron is measured via various indicators including: transferrin saturation, erythrocyte

protoporphyrin, mean cell volume, serum transferrin receptor, and hemoglobin concentration. Storage iron can be measured by total iron-binding capacity or serum ferritin.

Iron deficient erythropoeisis can be estimated using Hb and MCV. Hemoglobin is a protein in red blood cells that binds oxygen and delivers it to cells. It is the major carrier of iron in the body and thus describes the percentage of iron in the erythrocytes (Groff and Gropper 2000). Transient infections may potentially decrease hemoglobin; however, the decline is not likely to have any effect on developmental outcomes (Stoltzfus 2001). MCV is measured electronically in automated cell counters (Cook and Skikne 1989) from red blood cell distribution data (Van Hove et al. 2000). A low MCV (<70 fl) (Wu et al. 2002) is a specific marker of iron deficiency and can help assess the severity of iron deficiency if ferritin levels are below <12μg/L. In infants it is not uncommon to see microcytosis before anemia (Ulokol et al. 2004; Wright et al. 2004). MCV may also be depressed in infections (Zetterstrom 2004); although, a recent study evaluating parameters on sick infants and children with acute infections (URTI, GI, otitis, sinusitis, tonsillitis) showed no difference in MCV between sick and healthy children (Sipahi et al. 2004).

Hematocrit, which is the volume of erythrocytes in a venous sample, is commonly used to detect the presence or absence of anemia (Van Hove et al. 2000): Hct (<33) (AAP 2004). Mean corpuscular hemoglobin (MCH) relates to the concentration of hemoglobin in each erythrocyte. MCH has a direct linear relationship with MCV; mean corpuscular hemoglobin concentration (MCHC) is not a sensitive indicator for ID (Van Hove et al. 2000). MCH values in iron deficiency will normally fall before hemoglobin concentration (Wright et al. 2004).

Serum ferritin is an important measure of iron stores. Once ferritin is below <12μg/L (this cut-off indicates the depletion of stores), it offers no indication of the severity of iron deficiency. The caveat, however, is the uncertainty surrounding at what cut-off iron stores are completely exhausted in EBF infants. The measure of serum ferritin is further complicated by inflammation, because ferritin is an acute phase protein formed during simple infections and fever, and may be falsely elevated in such situations (Cook and Skikne 1989). To account for the presence of an inflammatory response and

consequently falsely elevated ferritin, C-reactive protein is measured. Erythrocyte sedimentation rate has been used in very few iron studies as a measure of acute inflammation (Garibay et al. 2001). ESR measures the suspension stability of red blood cells (Clarke 2004). ESR is influenced by the degree of agglutination of red blood cells and hematocrit and is not intended for screening of asymptomatic persons for an infectious process (Clarke 2004; Sox and Liang 1986). However, one study in infants found that CRP and ESR remained elevated 1-3 days after the start of an infection/illness and returned to their normal values after two weeks (Sipahi et al. 2004).

Currently many parameters are used to assess for ID but there is still much debate on which indicators are the most sensitive to iron deficiency and at what cut-offs. The gold standard for diagnosis of IDA is bone marrow biopsy. Since this is too invasive, hemoglobin response to iron treatment in anemic infants is used to diagnose IDA (Cooke and Skikne 1989).

FAO (2001) discusses that measures of iron metabolism overlap considerably in normal and iron deficient individuals; therefore, clinicians prefer to use multiple criteria to make the appropriate diagnosis. Using multiple criteria can increase the specificity (non-diseased subjects identified as such) at the expense of decreased sensitivity (diagnosis of individuals who are diseased). A reduction in sensitivity leads to underestimation of the true prevalence. Thus, FAO (2001) recommends using only serum ferritin as the indicator to assess iron nutriture. Although the true parameter (iron deficiency) may also be underestimated, it will be less than if multiple criteria are used (FAO 2001). There is a significant negative correlation between functional iron and serum ferritin levels below <12μg/L (Cook and Skikne 1989). Measuring hemoglobin (WHO 2001a) or serum ferritin remain a cost effective strategy for screening for anemia or iron deficiency in healthy populations (Cook and Skikne 1989).

(2) Adequacy of iron status indicators in infancy

In 13-month old British infants diagnosed with anemia, MCH and Hb showed the largest response to iron supplementation, which specifies that these two indicators may be specific markers for iron deficiency at this age (Wright et al. 2004). However, others (White 2005) have shown that hemoglobin concentration is a poor indicator of iron status in toddlers. The study by White (2005) examined the NHANES III data of American

children ages 12-35 months. Of the children diagnosed with anemia (Hb<110 g/L), only 30% were iron deficient. Most anemic toddlers were not iron deficient and most iron deficient toddlers were not anemic. Reducing the cut-off value for hemoglobin only made it a less sensitive marker of iron deficiency (White 2005).

Domellof et al. (2002) suggested new cut-offs for diagnosing iron deficiency since these reference values have not been well developed for infants. The authors argue that current cut-offs are based on the distribution of hematological parameters of children and may not be applicable to breastfed infants. The study examined two different populations, Swedish and Honduran exclusively breastfed infants enrolled from 0-3 months. Infants were recruited if the mother intended to EBF to six months or if the infant had been EBF from birth, were term, birth weight >2500g, and had no chronic illness (Domlloff et al. 2001). Infants were then randomized at 4 months into three groups: iron supplementation from 4-9 months; iron supplementation from 6-9 months; and placebo from 4-9 months. Hematological parameters were assessed at 4, 6, and 9 months of age. A normative population method was used to define cut-offs. Briefly, a normative population assumes that the population under study is healthy and may have low prevalence of ID. To ensure that infants who are ID are not selected, conventional cut-offs are used to eliminate those with ID. Consequently, an iron-replete normative population is obtained. Swedish infants at 4 months of age were the iron-replete population. Based on the mean parameters of the iron-replete population, the authors concluded that 2 SD cut-offs for Hb at four and six months should be <105 g/L, and 100g/L at nine months. At four months ferritin -2 SD cut-off should be at <20 g/L. The ferritin cut-off should be lowered from the current 10-12 g/L to <9 g/L at six months, and <5 g/L at nine months. The authors also concluded that Hb response to iron supplementation was inappropriate for diagnosis of iron deficiency anemia in infants under the age of six months. Possible reasons for immature or inadequate response of hemoglobin in this age group may be the presence of fetal hemoglobin (5%), which is regulated differently than adult hemoglobin (Domellof et al. 2002). Importantly, the relationship between these cut-offs and clinical disease outcomes of ID and IDA needs to be established (Domellof et al. 2002).

2.7. Conclusion

In developing countries exclusive or full breastfeeding can signify the difference between life and death (Beltran et al. 2001), health and infection (Kramer and Kakuma 2001), and adequate growth or stunting (Bhandari et al. 2003). As reviewed here, breastfed infants grow differently than formula fed infants, and exclusive breastfeeding reduces the incidence and severity of gastrointestinal infections; however, protection against respiratory tract infections remains inconclusive. Unfortunately, exclusive or full breastfeeding to six months is not commonly practiced in Mexico and breastfeeding is complemented with formula or solid food in early infancy. There is uncertainty regarding the impact that feeding habits have on infant health in poor, urban communities in Mexico with access to and routine use of local health services. The research study undertaken in Guadalajara, Mexico, and presented in the following section, describes, assesses, and examines how feeding can modify growth, illness, and iron status in infants followed from birth to six months of age.

3. Methods

Role of the MSc. Student In early 2003, Eva Monterrosa approached Dr. Noreen Willows to be her MSc. supervisor in international nutrition. Dr. Willows accepted under the condition that Eva was to find a project in Latin America. In April 2003, Eva contacted Dr. Edgar Vasquez, researcher and director of the Human Nutrition Institute at the University of Guadalajara, Mexico. Dr. Vasquez was willing to collaborate with Dr. Willows and Eva, but recommended meeting in Guadalajara to discuss the particulars of the project. Dr. Willows and Eva spent one week, July 23-30th 2003, in Guadalajara meeting students and faculty members, and touring the research facilities. During that visit, Dr. Willows, Eva, Dr. Vasquez, and Dr. Romero, a researcher at the Human Nutrition Institute, were able to define a suitable graduate project. From September 2003 to April 2004, Eva, with guidance from her supervisor and researchers in Mexico, was able to design a study (protocol, information sheet, consent forms, questionnaires), obtain ethics approval, and funding. In May 2004, Eva moved to Mexico while she completed her field work. Speaking fluent Spanish allowed Eva to function as an independent researcher and as a project manager. Duties included conducting all aspects of data collection (recruitment and follow-up), coordinating all follow-up visits, supervising the research assistant assigned to help out with the project, managing the budget ($30 000 CND), and conducting all administrative duties related to the project. In January 2005, Eva returned to the University of Alberta to complete coursework requirements for graduation. The project, which terminated in February 2005, was completed in her absence by her research assistant, Jissela Alfaro.

Preliminary results of this thesis were presented at a conference *10th Pediatric Nutrition Seminar* held in Guadalajara, Mexico from September 27-October 1 2005.

3.1. Study setting

The GMA of Guadalajara is comprised of 4 municipalities: Tlaquepaque, Zapopan, Tonalá and Guadalajara. The GMA of Guadalajara has a population of 4.5 million people making it Mexico's second most populous city. The Juan I Menchaca Hospital is one of two public, tertiary care hospitals which service the GMA of Guadalajara and the state of Jalisco. These two hospitals provide specialized medical care

at a reduced fee for individuals who do not have health insurance. In Mexico, those who do not have a stable job or are self-employed are unable to access hospitals in the National Health Insurance System of Mexico (IMSS as it is known in Mexico). Consequently, the type of population that seeks care at the Juan I Menchaca Hospital is low income. There are approximately 20 000 deliveries per year at this hospital and it is certified as a Baby Friendly Hospital.

3.2. Design:

Prospective, observational study of a convenience sample of mother-infant pairs recruited from the Juan I Menchaca Hospital in Guadalajara, Mexico. Infants were followed monthly from birth until six month of age.

3.3. Ethics Approval:

The study was approved by the Human Ethics Research Board, Faculty of Agriculture, Forestry and Home Economics, University of Alberta (Appendix 1), and by the Ethics Committee of the Juan I Menchaca Hospital, Guadalajara, Mexico.

3.4. Inclusion criteria:

A. Mothers:

- Primipara or second child for consistency in maternal characteristics and to compare breastfeeding inexperience with breastfeeding experience.
- Singleton birth, as multiple gestations normally result in smaller infants with reduced iron stores.
- 18 years old or older for legal purposes of signing the consent form
- living in the GMA of Guadalajara to ensure accessibility to the hospital

B. Infants:

- healthy, as determined by the pediatric note on the mother's chart
- term infants, 37 weeks of gestation or older as indicated on the mother's chart
- birth weight of $\geq$2500 g
- had not been separated from the mother since birth (rooming in)

3.5. Exclusion criteria

A. Mothers

- Mothers with a documented social or pathophysiological condition that would impair adequate care of the infant or interfere with the mother returning for subsequent visits:
  - Drug addiction or alcoholism
  - Congenital or mental disorders
- Anemia during pregnancy evidenced in the laboratory report by a moderately low parameter of hemoglobin (<100 g/L), or severe anemia requiring blood transfusion (<90 g/L) as stated on the mother's medical chart.
- Hypertension, diabetes, gestational diabetes, pre-eclampsia, eclampsia, or morbid obesity, HIV.
- Testing positive for Human Papiloma Virus (HPV) (women treated for urinary tract infections, yeast infections, or cervicovaginitis were included). HPV increases the infant's susceptibility to respiratory infections (Dr. Ileana Romo, Chief Gynecologist, personal communication).
- Moderate to severe oligohydramnios (poor placental volume: fetal growth may have been inadequate)

B. Infants

Sick infants, or those requiring extended hospital stays after birth were not considered for participation. Their inclusion would have increased the percentage of non-initiation or termination of breastfeeding in the neonatal period and increased the likelihood of reverse causality for breastfeeding. Those not included in the study:

- Apgar score of less than 7 at 5 minutes (indicative of hypoxia, breathing problems)
- Preterm, <37 weeks of gestation
- Low birth weight (<2500 g) as a proxy for IUGR
- Perinatal infection
- Congenital malformations

- HIV infection
- Metabolic disorders present at birth
- Left in the nursery under observation, or in an incubator (not rooming in)

3.6. Data collection

The author and a trained research assistant conducted all aspects of recruitment and follow-up. Figure 3 provides a schematic representation of the activities undertaken in recruitment and follow-up.

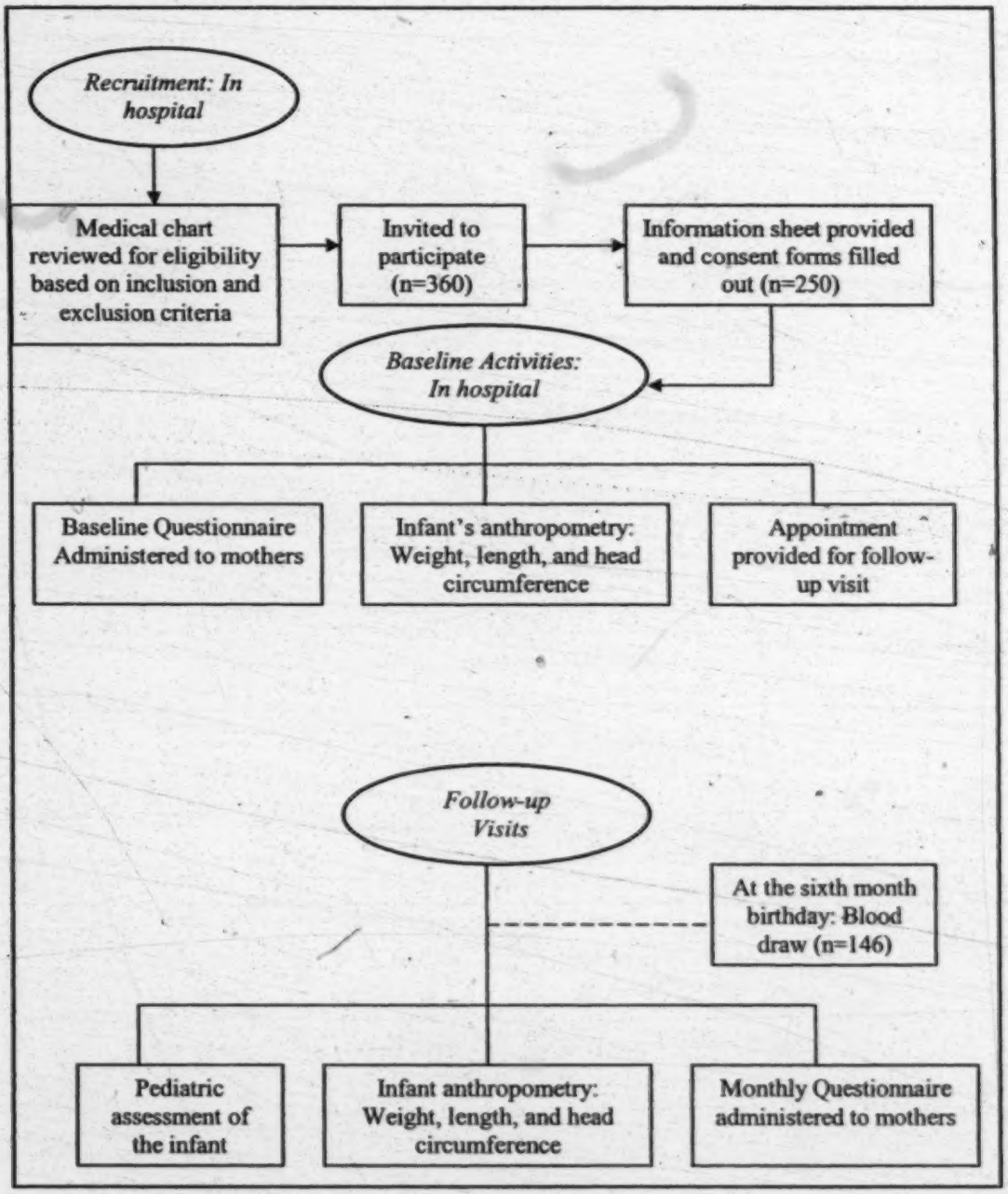

Recruitment: In hospital
Medical chart reviewed for eligibility based on inclusion and exclusion criteria
Invited to participate (n=360)
Information sheet provided and consent forms filled out (n=250)
Baseline Activities: In hospital
Baseline Questionnaire Administered to mothers
Infant's anthropometry: Weight, length, and head circumference
Appointment provided for follow-up visit
Follow-up Visits
At the sixth month birthday: Blood draw (n=146)
Pediatric assessment of the infant
Infant anthropometry: Weight, length, and head circumference
Monthly Questionnaire administered to mothers

Figure 3. Schematic presentation of the activities undertaken in data collection

A. Recruitment of participants

Recruitment took place from May-August 2004 while mothers were patients of the hospital following delivery. The maternity ward consists of 80 beds distributed on floors 5 and 6 of the hospital. Each hospital room is shared by a maximum of 6 women. The sixth floor is occupied by women who have vaginal deliveries, while the other floor is occupied by women who undergo a CS. Most of our recruitment took place on the sixth floor, not only because mothers were more receptive to our oral recruitment request but also were more likely to fulfill the inclusion criteria. Mothers who had undergone CS were more lethargic due to sedation, and were more likely to have complications during their pregnancy that would exclude them from participating in our study.

Researchers reviewed mothers' charts for eligibility. If a mother met the inclusion criteria, the study was briefly explained and she was invited to participate (appendix 2). Once the mother verbally acknowledged an interest in participating, the information sheet was provided and the study explained in more detail (appendix 3). The consent form was read to the mother and filled out by the researcher. Each mother signed the consent form (appendix 4). After receiving consent, the baseline questionnaire (appendix 5) was administered to the mother and infant's anthropometry was carried out by the researchers. Mothers received an appointment date for the follow-up visit in one month's time.

B. Monthly follow-up visits

Follow-up visits initiated in June 2004 and terminated in February 2005. Visits were scheduled ± one week from the monthly birthday. A total 953 follow-up visits were conducted. On average, 10-12 infants were scheduled each day, 4 days a week, between 9:00 am to 1:00 pm.

The visits were carried out in a large classroom at the outpatient clinic of the Juan I Menchaca Hospital and clinic fees of $75.00 ($8.00 CND) pesos per visit were waived. At these visits, infants' received physical assessments by the study pediatrician; the researchers measured infant anthropometry and administered the monthly questionnaire to the mothers (appendix 6). Normally the entire visit lasted about one hour. If it was the infant's second, fourth, or sixth month birthday, the mother was instructed to vaccinate the infant at the hospital. At the end of each visit, mothers were given a date for the next

follow-up and received their stipend. At the six month visit, venous blood was drawn and analyzed for iron nutriture and evidence of inflammation.

The pediatrician was responsible for assessment of physical health, adequacy of feeding, and appropriate weight gain since the previous visit. The pediatrician and researchers did not influence the mother's infant feeding decision; however, when answering a question regarding infant feeding researchers respected a mother's right to timely and pertinent medical/nutritional information. When a mother desired to use human milk substitutes, she was reminded of the recommendations (EBF to six months of age and introduction of solids thereafter) but also given the appropriate information as to the type and preparation of infant formulas.

Mothers were also encouraged to seek care with the study pediatrician when their child was sick. The rationale for doing so was to closely monitor illness.

C. Rationale for Stipend

Usually there is a high drop out rate in studies that involve monthly follow-up visits at clinics or hospitals (Friel et al. 2003). Though, monthly visits are emphasized, it is common for mothers to only attend well-baby clinics at the time of immunization (2, 4, and 6 months) (Dr. Marta Luna, Study Pediatrician, personal communication). There are many local health centres in the GMA of Guadalajara that provide this service thus negating the need to return to city hospitals. Given this situation, many mother-infant pairs would not have returned for follow-up at the Juan I Menchaca Hospital for one or more of the following reasons: commute time, baby was healthy, and/or community health centres providing the well-baby clinic service for a nominal fee. This loss to follow-up would have seriously biased the study. Because of the size of the city and difficulty in locating addresses, it was not practical or safe to provide in-home pediatric follow-ups. Therefore, to reduce the likelihood of a high drop out rate, all mothers were provided with a stipend of $200.00 pesos ($22.00 CDN) after completion of each follow-up visit. Mothers were not made aware of the stipend until the first visit. The stipend was to compensate for any costs incurred by the women for attending follow-up visits (transportation, and food).

D. Questionnaires

The baseline and monthly questionnaires were interviewer administered. Answers were coded based on the available responses. If a mother gave a response which was not available, the interviewer recorded the response and this response was subsequently coded by the author. When necessary, probing of participants was done in a neutral manner.

The baseline questionnaire was completed in the hospital room. This questionnaire consisted of 63 questions separated into 5 sections (appendix 5) and was intended to collect sociodemographic information on the mother and father of the infant. This information was used to describe our sample of mother-infant pairs and to control confounding variables in the statistical analysis. The questionnaire also included a section on prenatal care, previous breastfeeding experience, and intention to breastfeed. The baseline questionnaire was adapted mostly from Santos-Torres et al. (2003). Question 45 was adapted from Alaimo et al. (2001). Questions on housing characteristics were adapted from the UNICEF MICS Household Questionnaires (2003). The section on the mother and father sociodemographic data was adapted from Vasquez-Garibay et al. (2002).

The questionnaire used in the follow-up visits (appendix 6) was designed to capture information on infant feeding mode via food frequency and 24-hour recall, feeding advice provided to the mother, vitamins provided to the infant, symptoms of illness in the previous month, maternal employment and alternate care, and maternal smoking. The questionnaire was adapted from various sources including Santos-Torres et al. (2003), PAHO ProPAN Manual (2004), de Onis et al. (2004), and Rubin et al. (1991).

E. Anthropometric training of measurers

Both researchers (one of whom was Eva Monterrosa) were trained in infant anthropometry before the start of the study. Researchers standardized their measuring techniques to assure adequate intra- and inter-observer reliability for all three measurements (weight, length and head circumference). This was accomplished using a test-retest method, and testing researchers' measurements against that of an experienced measurer (Cogill 2003). Intra- and inter-measurements were all highly correlated ($r=0.9$).

F. Anthropometric measurements

Weight, length, and head circumference measurements were undertaken. Each anthropometric measurement was taken only once unless the infant was uncooperative and if it was suspected that the measurement was inaccurate. The reliability of weight, length, and head circumference between the two measurers was assessed periodically, though informally, throughout the study (once every two weeks). On average, the difference between the two measurers was 20g for weight, 0.3 cm for length, and 0.3 cm for head circumference. Anthropometric measurement techniques were those of the WHO (1995). Weight and recumbent length measurements were measured in nude infants. Specifically:

Weight: Weight was obtained to the nearest 10 gram on a pan beam balance scale (Health-O-Meter).

Length: Recumbent length was measured using a locally made measuring board. Two people were used to obtain recumbent length. Briefly, the infant lay in a supine position on the measuring board. One researcher positioned the infant's head ensuring that the crown of the head was perpendicular to the plane of the measuring board and the head was touching the headboard. Infant's hips and shoulders were at 90 angles to the long axis of their body. Shoulders, back, hips, knees and legs were flat against the base of the measuring board. To ensure this, the second researcher gently extended the infant's legs while bringing the vertical moveable board to the infant's feet. Length was recorded to the nearest 0.1 cm.

Head circumference: Hair accessories were removed from all infants. Head circumference was measured when the infant was either held or seated by one of the researchers or the mother. A steel measuring tape was placed around the middle of the forehead and the furthest protrusion of the occipital lobe. The measuring tape was pulled tightly enough to flatten the hair and the measurement was recorded to the nearest 0.1 cm.

G. Blood Analysis

Blood samples were taken at the infant's last visit (six month birthday). If infants demonstrated clear signs of illness, the blood draw was deferred until the following week or until the infant was convalescent. An experienced pediatric nurse drew blood via

venipuncture of an anterior vein in the hand. A total of 6 ml of blood were required for analysis. Three milliliters were deposited into a regular blood collection vial which did not contain EDTA for analysis of serum ferritin, and CRP. Three milliliters were collected into a vial containing EDTA for CBC. CBC was completed using Cell Dyne technology (Beckman Coulter) and serum ferritin was carried out using an immunoabsorbent assay (Beckman Immage). All laboratory parameters were determined using quantitative methods.

For the purpose of infant care, diagnosis of ID or IDA was done by the study pediatrician. Any infant identified by the study pediatrician to have ID or IDA was provided, free of charge, iron drops (Fer-in-sol) at amounts prescribed by the pediatrician. All of these infants returned for a subsequent blood draw to ensure that ID or IDA had resolved with supplemental iron treatment.

3.7. Analysis

A. Categorizing the data

Infant feeding

As recommended by Labbok and Karosev (1990), breastfeeding frequency was the basis for defining feeding categories used in the statistical analyses. Breastfeeding practices from birth to 4 months were used to group infants because feeding was most consistent in the first four months of life. At months 5 and 6, many infants switched feeding mode so that classifying infants into homogeneous and mutually exclusive groups was not feasible following 4 months.

The WHO (1991) criterion was used in the initial classification of infants into feeding categories; however, because very few infants were exclusively or predominantly breastfed, exclusive (n=5) and predominant breastfeeding (n=5) were amalgamated into one category called *primarily breastfed* (n=54). Also in this category were six infants that received breast milk complemented with infant formula in the first month of life but formula was not used in months 2-4. Primarily breastfed infants' main source of nutrition was breast milk but in addition some infants received water, teas, nutritive liquids (oat or rice water), $\leq$ 120 ml of formula/day, and/or the occasional 'taste' of solid food. The *Partial breastfed* category (n=38) were those infants who by the 4th month received

breast milk and formula (>120 ml of formula/day) as the main source of nutrition along with water, teas, nutritive liquids, daily amount of solid food, or powdered cow's milk (n=3). Infants classified as *formula fed* (n=50) were those completely weaned from breast milk to formula by the 3rd month. Of the formula fed infants, 13 received infant formula from birth, and 18 had been completely weaned from the breast by 2 months of age. *Any breastfeeding* (n=12) were those infants who had been weaned from the breast by 4 months and had received varying amounts of breast milk but did not classify into the other feeding groups. Some had skipped a visit in the 4 month period, so there was no knowledge of feeding that month. As there were few infants in this group, these infants were excluded from further analysis in comparisons among the feeding groups.

Growth

For descriptive purposes, infants were classified as stunted, wasted or underweight at six months of age by the WHO (1995) criteria. Growth was modeled as attained weight (g) and length (cm) at six months and as change in weight or length from birth to six months (measurement at six months – birth measurement). WHO/NCHS (1977) curves were used to compare the Z-scores of infants, as this remains the recommended reference population (Frongillo 2000b).

Infections

Because it is possible that 'breastfeeding may be protective against certain types of infections…and not others' (Bauchner et al. 1986 p.891), in the final analysis GI and URTI were analyzed separately as to not obscure a protective effect of breastfeeding if one were to exist in this population (Bauchner et al. 1986) . Rubin's algorithm (Rubin et al. 1991) was used to define GI and URTI infections. This algorithm had been used in a large study (PROBIT STUDY, Kramer et al. 2001) involving health outcomes and breastfed infants enabling comparison of results between that study and this one. Rationale for duration of symptoms was not to confuse symptoms of URTI or GI with possible allergies, or other undetected pathologies unrelated to study outcomes (Rubin et al. 1991).

Gastrointestinal infections Individual episodes of gastrointestinal infections were defined as having at least 2 symptoms with duration of 2-20 days: fever of 38.5 ℃, increased stool frequency, loose stools, vomiting, or diagnosis by our study physician. At least 3 symptoms were required for a diagnosis if no duration was reported. Diarrhea was not a specific endpoint; however, it was defined as increased stool frequency and/or loose stools. We decided against specifying the number of stools/day as breastfed babies and formula fed babies have different stool frequencies and consistencies. Mothers were asked if symptoms of increased stool frequency and/or loose stools were 'more than usual'.

Respiratory infections Respiratory tract infections were defined as having at least 2 symptoms with duration of 2-20 days: fever of 38.5 ℃, cough, fast breathing, rhinorrhea or diagnosis by our study physician. At least 3 symptoms were required for a diagnosis if no duration was reported.

Time-varying versions (0-6 months, 0-4 months, and 4-6 months) of GI and URTI infections were derived and tested against feeding mode for exploratory purposes. There were too few cases of GI and URTI in the first 3 months of life to statistically analyze and test for associations between feeding mode and infection. Most of the infection incidence occurred from 4-6 months. In the final analysis, only cumulative incidence (GI or URTI or both) in the first six months of life was used.

Iron status

Functional iron Anemia was defined as Hb <110 g/L (WHO 2001a). Microcytosis (MCV< 70 fl, Wu et al. 2002) may present itself before anemia in healthy infants. In our sample, microcytosis (6%) was not more prevalent than anemia (11%) and thus MCV was not used as an indicator of functional iron status.

Storage iron ID was defined as serum ferritin <12 μg/L (WHO 2001). Since ferritin is elevated during acute inflammation, CRP >0.8 mg/L was considered a marker for acute inflammation based on the laboratory specific cut-off. In infants with elevated CRP (n=10), ferritin concentration was not significantly different from infants with normal CRP (p=0.56). For this reason, we did not exclude infants who had elevated CRP

from the analysis of iron status. Minimum and maximum values for CRP were 0.1 mg/L and a maximum 2.9 mg/L, respectively. Mean (SD) CRP values was 0.3 ± 0.4 mg/L.

Because of the current controversies surrounding infant cut-offs for serum ferritin and hemoglobin, various inferior limits were tested for assessment of iron deficiency and anemia prevalence. In addition to the criteria specified, we also used cut-offs suggested by Domeloff et al. (2002a) (ferritin <9 μg/L, and Hb <105 g/L) and Wu et al. (2002) (ferritin <10 μg/L).

3.8. Statistical analyses

A. Conceptual Model

The conceptual model (appendix 7) provides a detailed description of the relationship of the various variables on the outcomes of interest. The predictor variable was infant feeding practices from 0-4 months. The outcome variables were growth (weight and length gain), cumulative incidence of infection in the first six months of life, and iron status at six months (iron depletion, ID, anemia, and IDA). The covariates designated as maternal, household, and infant are all fixed variables that were measured at baseline. Their inclusion in the conceptual model is to control for factors that may directly influence infant growth, illness and iron status or indirectly through feeding practices. The majority of the variables were included in the initial analyses and excluded when they did not significantly affect the statistical model ($p>0.5$) (Frongillo 2005).

B. Statistical procedures and models

Growth data was entered into EpiInfo 3.2.2 (CDC, Atlanta, GA, USA) to obtain Z-scores, and then exported to SPSS Version 13 (SPSS Inc. Chicago, IL, USA). All variables were entered and analyzed using SPSS. Results from categorical variables were presented as frequencies and percents, and results from continuous variables were presented as means ± standard deviations. A p-value of <0.05 was considered significant for inferential statistics. Categorical covariates used in all statistical procedures were converted into dummy variables (appendix 8). Ferritin, which was positively skewed, was transformed into ln (natural logarithm) ferritin to use in ANOVA, Regression and T-tests. Geometric means were provided for ln ferritin.

ANOVA and the independent sample t-test were utilized to assess differences in means. Comparisons among feeding groups were carried out using ANOVA with Tukey's post-hoc test. Linear regression was employed to test for associations among the feeding categories with weight, length, Z-scores, ferritin, and Hb.

Iron deficiency, anemia, and health status were analyzed using Chi-square test, and logistic regression. Unadjusted and adjusted odds ratios were presented (e^b). Adjusted odds ratios were used to express the probability a covariate had on the disease state while holding all other variables constant. Confidence intervals for adjusted odds ratios were also reported. All conceptually significant covariates were included in the logistic regression models. Various models were executed and the best one selected. Suitability of the model was based on (Frongillo 2005):

1. a significant Chi-square test for the overall model with covariates
2. A significant improvement in the -2 log likelihood ratio when covariates were removed.
3. R^2 (Garson 2005)

4. Results

The statistical analyses presented in this section were specific to the objectives:

1. For all the infants in the cohort, describe growth and cumulative incidence of infection (GI and URTI) from birth to six months, and iron status at six months.
2. Explore the relationships between infant feeding practices (predictor variable), and outcome variables while controlling for significant covariates.

   a) Compare changes in growth, cumulative incidence rates of infection (GI and URTI) and iron status across infant feeding groups.

The results presented herein answer the two research questions:

1. How do infant feeding practices influence infant growth and illness from birth to six months, and iron status at 6 months of age?
2. What are the trade-offs among feeding practices on illness in the first six months and iron status at 6 months of age?

4.1. Response rate

Of the mothers eligible to participate 70% agreed to do so. Reasons for not participating included: mother lives too far from hospital, mother returning to work, mother moving away soon, infant already has a pediatrician, mother has another child and unable to manage both, or mother prefers to discuss joining the study with her husband before agreeing to do so. Some mothers did not provide a reason for not participating.

4.2. Drop-out rate

A total of 250 mother-infant pairs were recruited in hospital; 165 dyads initiated follow-up at one month post-partum; 154 completed the study, and 146 infants had their blood drawn. Table 1 describes the percent drop-out rate for different phases of the study.

Table1. Dropout rates at different phases of the study[1]

Period of Interest	Number of Infants	Dropout rate from recruitment	Dropout rate from follow-up
Recruited	250	-	-
Initiated follow-up	165	34%	-
Followed from 0-4 months	158	36.8%	4.2%
Followed from 0-6 months	154	38.4%	6.7%
Infants with blood data	146	41.6%	11.5%

The main reason for the initial loss to follow-up was being unable to remind the mother of her appointment because of no phone service, or wrong number. Other reasons provided by the mothers for not continuing in the study were long commute time, moving away, and seeing another pediatrician. Those infants who were followed for six months but did not have blood drawn (n=8) were sick at the six month visit. Despite rescheduling their appointment for blood draw and contacting the mother to remind her of the appointment, these mothers chose to not return for the blood draw for unknown reasons.

4.3. Dropout analysis

Dyads that did not complete the study (n=96) did not differ from participants (n=154) in birth weight, birth length, sex of infant, feeding mode, parity, household income, or age of mother. Dropouts were less educated (p=0.03), more likely to be in a common law relationship (p=0.025), and were more food insecure (p=0.038).

4.4. Characteristics of participants

Mothers Mothers in the study received adequate prenatal care. Ninety-seven percent (n=150) reported seeing a doctor, while 80% (n=123) had visited the doctor 4-5 times throughout their pregnancy. However, only 31% (n=47) stated to have received information about breastfeeding from a physician/nurse prior to delivery. Supplement

use during pregnancy was high: 92% (n=142) reported using iron and 87% (n=134) reported using prenatal vitamins. The majority of the mothers had vaginal deliveries (89%, n=137).

Relevant maternal and household characteristics of the dyads that completed the study (n=154) are presented in Table 2.

Table 2. Maternal and household characteristics[1,2]

Characteristics	
Age* *years*	21.7 ± 3.3
Height* *cm*	157.6 ± 6.11
Parity	55% (85) first child, 45% (69)second child
Education	63% (97) completed 9 or more years of school
Marital Status	25% (37) single, 40% (62) common law , 35% (54) married
Household income	18.2% (28) earned less than 2480 pesos/month ($270 CDN)
Food expenditure	94% (144) spent more than 1000 pesos/month ($109 CDN)
Food security	5% (8) were food insecure
House ownership	55% (86) live with their parent or in-laws, 25.3% (39) rented their home, 6% (9) borrowed their home from a relative, 13% (20) owned their homes
People in the home**	5

[1]n=154, except height: 7 measurements were missing; [2]expressed as % (n) unless otherwise stated.
*expressed as mean ± standard deviation;**expressed as median

The majority of the mothers reported a household monthly income between 2481-4960 pesos ($270-$540 CDN) (70%, n=109). Despite being low income, 91% (n=140) had a refrigerator, 98% (n=151) had sewage disposal and 99% (n=152) had a gas cooking stove.

Of the mothers that choose to provide infant formula, 64% (n=32) reported using boiled bottled water and sterilized bottles and teats throughout the six month period. The

rest of the mothers (30%, n=18) reported using boiled tap water or bottled water and sterilized bottles and teats. Use of unboiled tap water for preparation of formula was not reported (n=0).

Infants Of the 154 infants who completed the study, 53% (n=82) were boys and 47% (n=72) were girls. Mean birth weight was 3109.0 ± 349.2 g. Birth weight was not significantly different between boys and girls. At the time of recruitment 93% (n=143) of infants had initiated breastfeeding and of these, 33% (n=52) had done so within two hours of birth. Only 30% (n=45) of infants were primarily breastfed to six months. On average, breastfeeding was terminated at 4.5 months.

Of the 154 infants who completed the study, six mothers (4%) reported that their infants had received iron supplements on a daily basis. Of these six infants, two received iron supplements from 3-6 months and the other four infants received only one month of iron supplementation. All infants who received iron supplementation on a daily basis belonged to the *formula fed* category.

4.5. Predictor variable

Infant feeding practices from 0-4 months was the predictor variable. Infants in the *any breastfeeding* (n=12) category were included in all descriptive statistics but excluded from further analyses that required comparisons among the feeding groups.

Infant feeding did not differ by infant sex, infant birth weight, maternal education, maternal age, maternal height, income, or parity (data not shown). Infants who were primarily breastfed were more likely to come from a food insecure household (p=0.01, Fisher's exact test).

In the first four months, formula fed infants received on average 870 ± 219 ml of iron-fortified formula daily; whereas, partially breastfed infants received 432 ± 240 ml of iron-fortified formula daily. The mean difference of 396 ml (95% CI: 339, 456) in formula intake between the two groups was statistically significant (T-test p<.0001). All infants who were fed with formula received iron-fortified formula. All iron-fortified formulas provided to the infants contained, on average, 9 mg/L of iron (8-12 mg/L). Some infants in the formula fed category received iron-fortified formulas supplemented with AA (arachadonic acid) and DHA (docosahexanoic acid) (22%, n=11). Of these 11

infants, five received the EFA (essential fatty acids) formulas for 5-6 months, five received the EFA formulas for 3-4 months and only one infant received EFA formula for 1 month.

To verify that primarily BF infants received significantly more breast milk than the other three feeding groups, mean duration and frequency of breastfeeds were calculated and are presented in Table 3. Mean duration of breastfeeding was comparable between primarily and partially breastfed infants; however, partially breastfed infants received significantly less breastfeeds per day. The high variability in duration and frequency of breastfeeds confirmed the heterogeneity of the *any breastfeeding* group.

Table 3. Infant feeding method from birth to 4 months: mean duration and frequency of breastfeeding

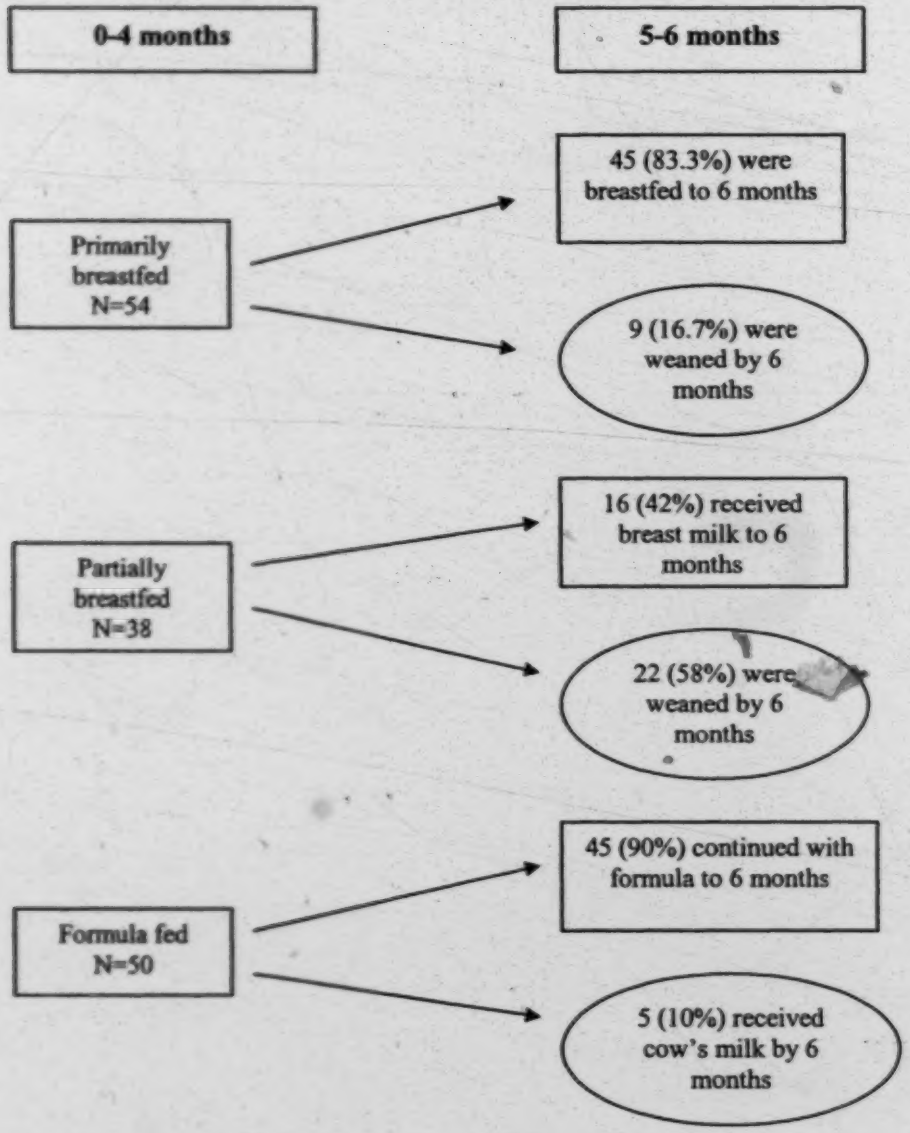
Feeding method	Percent (n)	Number of boys in the group % (n)	Duration of BF in months Mean (SD)	Daily Frequency BF° Mean (SD)
Primarily breastfed	35.1% (54)	50.0% (27)	3.9 (0.3)	10.6 (2.6)
Partially breastfed	24.7% (38)	60.5% (23)	3.9 (0.2)	7.8 (3.0)†
Formula fed	32.5% (50)	56.0% (28)	1.3 (0.9)*	9.3 (4.9)
Any breastfeeding	7.8% (12)	33.3% (4)	3.3 (1.0)*	8.8 (4.7)
Total	100% (154)	100% (82)		

° mean daily frequency of breastfeeds for the mean duration of breastfeeding. Derived by tallying the daily frequency of breastfeeds for the interval of time each infant was breastfed

† significantly less than primarily breastfed: T-test, $p<0.001$; * significantly different from primarily breastfed: ANOVA with Tukey's post-hoc test, $p<0.005$

To further illustrate the feeding differences between *primarily breastfed* infants and *partially breastfed* infants, Figure 3 presents the breastfeeding patterns of the groups after 4 months. Infants classified as partially breastfed were more likely to be weaned between 5-6 months (58%) versus infants who were primarily breastfed from birth (16%).

Figure 4. Breastfeeding practices after four months of infants who completed the study.


0-4 months
5-6 months
Primarily breastfed N=54
45 (83.3%) were breastfed to 6 months
9 (16.7%) were weaned by 6 months
Partially breastfed N=38
16 (42%) received breast milk to 6 months
22 (58%) were weaned by 6 months
Formula fed N=50
45 (90%) continued with formula to 6 months
5 (10%) received cow's milk by 6 months

4.6. Outcome variables

A. Growth

Table 4 presents weight and length at birth, at six months, and change in growth from birth to six months.

Table 4. Infant growth from birth to six months

Variable (mean ± SD)	**Cohort** (n=154)	**Boys** (n=82)	**Girls** (n=72)
Birth weight (g)	3110 ± 350	3150 ± 340	3060 ± 360
Weight at 6 months (g)	7410 ± 890	7700 ± 930*	7080 ± 730
Weight gain (g)	4300 ± 800	4550 ± 840*	4020 ± 660
Birth length (cm)	48.8 ± 1.6	49.2 ± 1.6*	48.3 ± 1.5
Length at 6 months (cm)	65.5 ± 2.3	66.5 ± 2.2*	64.3 ± 1.8
Length gain (cm)	16.7 ± 1.9	17.3 ± 1.8*	16.0 ± 1.7

*Significantly different from girls, $p<0.001$

Of the 154 infants who completed the study, all but ten (6%) doubled their birth weight by six months of age (8 girls and 2 boys). Of the ten infants, two were BF and eight were not BF. Table 5 shows average growth of infants in the three feeding groups. There were no significant differences among the groups.

Table 5. Average growth from birth to six months, sex, and maternal height according to the feeding mode

Growth variable (mean ± SD)	**Primarily breastfed** (n=54)	**Partially breastfed** (n=38)	**Formula fed** (n=50)
Birth weight (g)	3050 ± 320	3200 ± 360	3140 ± 370
Attained weight (g)	7310 ± 930	7570 ± 930	7480 ± 850
Weight gain (g)	4260 ± 800	4370 ± 930	4330 ± 760
Birth length (cm)	48.5 ± 1.6	49.2 ± 1.6	48.8 ± 1.5
Attained length (cm)	65.1 ± 2.3	65.9 ± 2.6	65.7 ± 2.6
Length gain (cm)	16.6 ± 1.8	16.7 ± 2.1	16.9 ± 1.9
Number of boys	50% (n=27)	60.5% (n=23)	56% (n=28)
Maternal height (cm)	156.9 ± 6.6	159.2 ± 6.5	156.8 ± 5.4

Inferential Statistics Gastrointestinal infections and upper respiratory tract infections (ever sick) did not influence attained weight or length at six months, or changes in weight and length ($p>0.5$) and illness was removed from the final models.

Covariates included in the final model for attained weight at six months were maternal height, sex, infant feeding, and birth weight (adjusted $R^2 = 0.279$; $F(5, 130) = 11.5$, $p<0.001$) . Attained weight was not associated with feeding mode ($p>0.7$). Significant predictors of weight at six months were maternal height, sex, and birth weight. A one centimeter increase in a mother's height was associated with 29.4 g more in attained weight ($p= 0.009$), and boys were 543 g heavier than girls ($p<0.001$). Birth weight was by far the strongest predictor of attained weight. For every 500 g increase in birth weight, infants were 472 g heavier at six months ($p<0.001$).

Covariates included in the final model to test for associations with attained length at six months were feeding method, maternal height, sex, and birth length (adjusted $R^2 = 0.462$; $F (5, 130) = 24.2$ $p<0.001$). Infant feeding was not associated with attained length ($p>0.5$). Although maternal height and birth length were significant predictors of infant length, their effects on length at six months were minor. A one centimeter increase in a mother's height added a negligible 0.05 cm in attained length ($p=0.04$). A one centimeter increase in birth length was associated with 0.8 cm more in attained length at six months ($p<0.001$). Similar to attained weight, sex was a strong predictor for length: boys were 1.5 cm longer than girls ($p<0.001$) at six months.

To evaluate changes in weight and length from birth to six months, regression analysis was completed using the same model as attained weight. Weight gain in the first six months was significantly associated with sex of infant and maternal height (Table 6). Length gain ($F(4,130) = 5.17$, $p<0.001$, adjusted $R^2 = 0.110$) was not significantly associated with any covariates other than sex (boys being longer than girls by 1.3 cm; $p<0.001$) (data not shown). Neither weight nor length gain were associated with infant feeding. Other household or maternal covariates were not associated with growth during this time period (data not shown).

Table 6. Linear regression model for weight gain (g) from birth to six months[1]

Variable	B coefficient*	P value
Mother's height	29.9	0.009
Sex of infant (boys)	539.4	0.000
Primarily breastfeeding†	-43.3	.695
Partial breastfeeding†	-67.4	.782

[1]n=135; *any breastfeeding* excluded (n=12); missing mother's height for 7 infants
*unstandardized coefficients Linear regression, $F(4, 130) = 6.09$; $p<0.001$; $R = .396$ adjusted $R^2=0.130$
†When compared to formula fed infants

At birth all anthropometric indices were below the mean of the WHO/NCHS (1977) growth curves. At six months WAZ and LAZ remained below the WHO/NCHS reference mean. Mean WAZ and LAZ at six months did not differ between the sexes or among feeding groups. On average, changes in WAZ, WLZ, and LAZ from birth were positive. Only one child was underweight and three were stunted at six months. Of the three infants that were stunted at six months, two were breastfed and the other one was formula fed. The underweight infant at six months was primarily breastfed. Table 7 presents mean Z-scores for the entire cohort. There were no statistical significant differences among the groups in mean Z-scores at birth, six months, or change in Z-scores from 0-6 months among the feeding groups.

Table 7. Mean Z-scores at birth, six months and from birth to six months[1, 2]

Time point	WAZ	LAZ	WLZ
Birth	-0.36 ± 0.78	-0.68 ± 0.71	-0.19 ± 0.67*
At six months	-0.17 ± 0.88	-0.57 ± 0.77	0.29 ± 0.89
Change from 0-6 months	0.19 ± 0.88	0.12 ± 0.69	0.36 ± 1.07*

[1]n=154. *Birth and Change WLZ (n=70). Values only reflect change in 70 infants
[2]Presented as mean ± SD using WHO/NCHS (1977) growth charts.

B. URTI and GI

Table 8 shows cumulative incidence of infection for GI and URTI. There were no differences in the frequency of URTI or GI among the feeding groups (data not shown); therefore, all incident cases were collapsed into one group *ever sick*. Table 9 shows that the likelihood of having at least one infection (URTI and GI) was not different among the three feeding groups.

Table 8. Incidence of infection in the cohort from birth to six months[1]

Type of infection	Not sick ever	One infection	Two infections	At least 3 infections
Gastrointestinal	71.4% (n=110)	23% (n=35)	4.5 % (n=7)	1.2% (n=2)
Upper respiratory tract	34.4% (n=53)	35.7% (n=55)	20% (n=31)	9.7% (n=15)

[1]n=154

Table 9. Ever sick infants with GI or URTI stratified by feeding group[1]

Feeding group	Ever sick	
	GI	URTI
Primarily breastfed	20.4% (n=11)	65% (n=35)
Partially breastfed	37% (n=14)	66% (n=25)
Formula fed	34% (n=17)	68% (n=34)

[1]n=142, *Any breastfeeding* excluded (n=12)
GI $X^2 = 3.63$, p= 0.163; URTI (X^2 =0.122 p= 0.941)

To test the assumption that breastfeeding is protective against illness in early infancy, primarily breastfed and partially breastfed infants were combined into one group, *breastfed*, and compared against *not breastfed* (formula fed infants). Having at least one case (ever sick) of URTI (data not shown) or GI was not different between breastfed vs. not breastfed infants (Table 10).

Table 10. Ever sick from a GI since birth between breastfed and not breastfed infants

Feeding categories	**Gastrointestinal infection**[1] *Percentage* (n)	
	Yes	**No**
Breastfed	*27.2* (25)	*72.8* (67)
Not breastfed	*34.0* (17)	*66.0* (33)

[1] Fischer's Exact test one sided p=0.254; n=142. *Any breastfeeding* excluded (n=12)

Because intensity and duration of breastfeeding is linked to protection against illness, partial breastfed and formula fed groups were combined into one group, *not primarily breastfed,* and compared against *primarily breastfed* infants. Having at least one case of URTI from birth to six months was not different between primarily BF versus not primarily BF infants (data not shown). Having at least one GI infection was significantly less among primarily BF infants than those who were not primarily BF. Unadjusted odds ratio (CI) for having at least one GI infection if not primarily BF was 2.12 (0.96, 4.70) (Table 11). GI was not associated with any of the household (number of children in the house, house ownership, and refrigerator) or maternal covariates (education) included in the final model (data not shown). Adjusted odds ratio for at least one GI if not primarily BF was: 2.41 (1.05, 5.56). Thus, it appears that high intensity and duration of breastfeeding protects against GI infection, not breastfeeding *per se*.

Table 11. Ever sick from a GI since birth between primarily breastfed and not primarily breastfed infants

Feeding categories	**Gastrointestinal infection**[1] *Percentage* (n)	
	Yes	**No**
Primarily breastfed	*20.4* (11)	*79.6* (43)
Not primarily breastfed	*35.2* (31)	*64.8* (57)

[1] Fischer's Exact test one sided p=0.044; n=142. *Any breastfeeding* excluded (n=12)

C. Iron status

Serum ferritin and hemoglobin Table 12 presents the mean hemoglobin and serum ferritin concentrations for the feeding groups and the cohort. For the entire cohort, mean hemoglobin concentration was 119 ± 9 g/L (n=146), which is above the cut-off (Hb<110 g/L) for anemia. Mean hemoglobin concentrations differed significantly only between BF and FF infants. The mean serum ferritin concentration for the entire cohort was 54.9 µg/L, the median was 41.2 µg/L, and minimum and maximum values were 4.70 µg/L and 226.5 µg/L, respectively. BF infants had significantly lower mean serum ferritin concentrations than FF infants ($p<0.01$). Neither serum ferritin nor hemoglobin concentration was influenced by GI or URTI (data not shown).

Table 12. Mean hemoglobin and serum ferritin concentrations by feeding group[2]

Group	Hemoglobin Concentration (g/L) (n=135)	Serum ferritin concentration (µg/L) (n=134)
Primarily breastfed	116 ± 8*	27.1*
Partial breastfed	120 ± 9	40.4
Formula fed	122 ± 9	52.3

[2]Presented as mean ± SD. Geometric means are presented for serum ferritin
* ANOVA, Tukey's post-hoc test, compared to formula fed infants, $p<0.005$

To see the effect of infant feeding on iron stores while controlling for other potential confounders, a regression analysis was employed with serum ferritin as a continuous outcome variable. Included in the final model for serum ferritin concentrations were parity, infant feeding, household income, birth weight, weight at six months, and sex (adjusted $R^2 = 0.342$). Covariates significantly associated with higher odds of lower serum ferritin concentration at six months of age were: infants living in household earning more than $260 CDN/month (OR=1.2); boys (OR=1.4); primarily breastfed infants (OR=1.9); and infants with higher attained weight (OR=1.4). Parity and partial breastfeeding were not significantly associated with lower serum ferritin concentrations at six months of age. The model is presented in Table 13

Table 13. Linear regression model for serum ferritin concentrations at six months of age[1]

Variable	Serum ln ferritin Beta Coefficients*	Adjusted Odds ratio (CI)	P-value
Parity (2nd child)	0.205	1.2 (1.0, 1.57)	0.11
Income (higher income)	-0.418	1.5 (1.1, 2.1)	0.015
Sex (boys)	-0.305	1.4 (1.1, 1.8)	0.021
Breastfeeding†	-0.658	1.9 (1.5, 2.6)	0.00
Partial breastfeeding†	-0.260	1.3 (1.1, 1.8)	0.10
Birth weight (500 gram increase)	0.508	1.7 (1.2, 1.4)	0.00
Attained weight (kg)	-0.340	1.4 (1.1, 1.3)	0.00

[1] n=134;

*unstandardized coefficients; odds ratio: e^b Ferritin model: F (7, 126) = 10.892, $p<0.0001$; R= 0.614, adjusted $R^2 = 0.342$)

†when compared to formula fed

A regression analysis was also employed with Hb concentration as a continuous outcome variable using the same covariates in the ferritin model; however, only breastfeeding (compared to formula feeding) was significantly associated with Hb concentration (b=-5.1, p=0.009). The overall model was not significant (F (8, 127) = 2.03, p=0.048; adjusted R^2= 0.06). Furthermore, very little of the variability was explained by the model.

Iron deficiency and anemia Overall, prevalence of iron deficiency at six months was low. Table 14 describes the prevalence of iron deficiency and anemia in the entire cohort using various cut-offs. Using the WHO criteria for screening in a healthy population, more infants were anemic (12.3%) than iron deficient (9.7%). Only one infant presented IDA as defined by the WHO criteria of Hb <110 g/L and ferritin <12 μg/L. Of the 14 infants who were iron deficient (ferritin <12 μg/L), four (29%) were girls and ten (71%) were boys (p=0.170). All four girls were breastfed; whereas six boys were breastfed and four were partially breastfed.

Table 14. Overall prevalence of iron deficiency and anemia at six months for the entire cohort[1]

Cut-offs	*Percentage* (n)
Iron deficiency: serum ferritin	
WHO: <12 μg/L	*9.7* (14)
AAP: <10μg/L	*8.3* (12)
Domellof[•]: <9μg/L	*7.6* (11)
Anemia: hemoglobin	
WHO and AAP:<110 g/L	*12.3* (18)
Domellof[•]: <105 g/L	*4.1* (6)

[1]N= 146; [•]Domellof et al. J. Nutr 2002a; 132: 3680-86.

Table 15 describes the overall prevalence of ID and anemia among the feeding groups using various cut-offs. FF infants had no evidence of ID. Because assumptions of the chi-square statistic were not met, the difference in prevalence of ID between formula fed and breastfed infants was not reported. Although there were differences in prevalence of anemia among the feeding groups, these differences did not reach statistical significance.

Table 15. Prevalence of iron deficiency and anemia using various cut-offs at six months according to feeding group

Feeding group	Iron deficiency % (n)[1] (serum ferritin)			Anemia %(n)[2] (Hemoglobin concentration)	
	WHO <12μg/L	AAP <10μg/L	Domellof <9μg/L	WHO/AAP* <110gL	Domellof <105g/L
Primarily breastfed	20 (10)	18.0 (9)	18.0 (9)	14.0 (7)	4.0 (2)
Partially breastfed	10.8 (4)	8.1 (3)	5.4 (2)	18.4 (7)	5.3 (2)
Formula fed	0 (0)	0 (0)	0.0	4.3 (2)	2.1 (1)

[1] n=134 [2]n=135. *Any breastfeeding* excluded (n=12) *Trend for differences among groups X^2, p= 0.112

Domellof et al. J. Nutr 2002a; 132: 3680-86.

To test the hypothesis that infants at highest risk for ID are those that are breastfed, iron deficiency was analyzed as a function of receiving primarily breast milk (*primarily breastfed*) versus receiving varying amounts of formula (*not primarily breastfed*: partially breastfed and formula fed combined). Unadjusted odds ratio for ID (ferritin <12 μg/L) in primarily BF group was 5.0 (1.49 - 16.95) (20% vs. 4.8%), (Fisher's exact test, p<0.008). When other covariates were included in the model (sex and parity) primarily breastfeeding remained a significant risk factor for iron deficiency at six months, adjusted OR= 6.28 (1.75, 22.72) (Table 16).

Table 16. Logistic regression model for iron deficiency (serum ferritin <12 μg/L) at 6 months

Covariate	Adjusted Odds ratio	P-value
Parity (primiparous)	2.7 (0.7 ,9.9)	0.136
Sex (boy)	3.2 (0.9, 11.8)	0.082
Primarily Breastfeeding	6.3 (1.8, 22.7)	0.005

X^2=12.6, p=0.005; R^2 =0.184 Log likelihood -77.30

5. Discussion

5.1. Major findings

This study reports the current breastfeeding practices from 0-6 months in infants living in low income households in Guadalajara, who were born at the Juan I Menchaca Hospital. This study found no evidence that breastfed and formula fed infants grew differently from birth to six months. Gain in weight and length, attained weight and length, and mean WAZ, LAZ and WLZ were not different among the feeding groups. Furthermore, cumulative incidence of URTI was comparable among the feeding groups. However, infants who were not primarily breastfed were more likely to have one or more gastrointestinal infection than primarily breastfed infants. Although at six months the prevalence of iron deficiency and anemia was low overall in the cohort, the study reported that mean serum ferritin concentrations were lower in primarily breastfed infants and that these infants were at higher risk for ID at six months.

5.2. Strengths and limitations of the study

Strengths A stringent inclusion criteria helped assure that the outcome variables were related to the predictor variable and not to other unmeasured conditions that may have influenced growth, infection, and iron status. A prospective design and timely, consistent follow-ups helped eliminate detection bias. The study design allowed certainty in reporting associations because the infants were free of detectable illness or pathology at the beginning of the study and outcomes were recorded as they occurred in time. Strict diagnosis criteria were employed to assure that sick infants were classified as such. Feeding practices were not assessed by a 24-hr recall (current feeding status method) instead by what happened in the entire feeding period; therefore, feeding practices reported in this study are better estimates of true infant feeding habits. Furthermore, the MSc. student was involved in all aspects of the study; thus data were collected in a consistent manner.

Limitations Because this was a convenience sample of mother-infant pairs, there was a selection bias towards mothers with adequate child caring practices to join the study; thus the results may not be extrapolated to all Mexican infants in urban low-income populations. One month's time may have been too long of a recall period and

therefore maternal recall bias may have influenced the results. Also, maternal reports of feeding and symptoms of illness are subject to both overreporting and underreporting bias.

Because, infant feeding was measured from 0-4 months and the outcome variables were measured from 0-6 months, there were two months where feeding habits were not accounted for. However, we were able to document how infants were fed from 5-6 months, and we know that there was little change in feeding type, with the exception of the partially breastfed group. It is not known for certain how the relationship among feeding, growth, infection and iron status would have changed had we accounted for infant feeding from 5 – 6 months. It is unlikely that growth or incidence of URTI would have been significantly influenced in the 5-6 month interval; however, it is plausible that the relationship between infant feeding practices and iron status was attenuated. For example, the use, or increased use of iron-fortified formula from 5-6 months may have potentially increased iron stores in partially breastfed infants and breastfed infants. Also, the strength of the relationship between BF and reduced GI infection may have been diminished due to reduced breastfeeding in some infants in the 5-6 month interval.

5.3. Power of the study

Power is the ability to detect a statistical difference in the population assuming that a difference is there to begin with. Assuming a ß of 0.8 and a of 0.95, the study had sufficient power to detect a difference of 2 g/L in hemoglobin concentration and 12 μg/L in serum ferritin between BF and FF groups. Also, only 22 infants per group were needed to detect a true prevalence of iron deficiency (<12 μg/L). Fifty-two infants per group were required to detect a difference of 162 grams in attained weight observed between BF and FF infants. We had sufficient power to detect the differences observed in incidence of GI (16 infants per group). To detect a reduction of URTI incidence of 25% in BF infants, only 11 infants were required per group.

The study lacked sufficient power to detect a differential weight gain of 105 grams observed between formula fed and breastfed groups. Whether the differences in attained weight and weight gain among groups are of clinical importance is debatable,

particularly since the differences were less than what is normally expected for measurement error (240 g).

5.4. Drop-outs

Because the study required mothers to return to the hospital for follow-up, a high drop-out rate was expected. Drop-out rate from recruitment was 40%; however, the majority never returned to the first follow-up. Friel et al. (2003) reported a 34% drop-out rate at six months in a prospective cohort of Canadian infants.

Dropouts were less educated and were more food insecure. Other than food insecurity, dropouts did not differ from our participants in measured covariates.

5.5. Participants' characteristics

Since this was a convenience sample, women who participated were most likely different from those who did not. Mothers were not aware that they would receive a stipend until the first follow-up visit; therefore, it is likely that those women who elected to continue in the study did so in the pursuit of optimal child care. Thus, it can be speculated that one of the differences between participating and not participating mothers might have been infant care practices. For instance, completing the study may be a trait of women who actively seek medical care, and raise healthy infants despite economic constraints. Zietlin (1991) terms these types of care practices as maternal technology in favour of positive health outcomes. Maternal technology, as describes by Zietlin (1991), includes the use of health services, good hygiene, safe food preparation, and adequate care during illness. Some indirect measures of maternal technology captured in the study include a high rate of prenatal care, the use of prenatal vitamins and iron, and appropriate preparation of infant formulas (using boiled bottled water, and sterilizing bottles and teats). Although not reported in this thesis, mothers with sick infants often sought care with other doctors in addition to the study pediatrician.

Furthermore, child development and health is influenced, but not limited to, the immediate environment and social system (Engle et al. 1996). Positive family dynamics, support from extended families, neighborhoods, and even formal marriages may protect infants against environmental stresses (Engle et al. 1996). For example, having one child

or no children may be a marker for infant care (the more children in the household, the less time each child has with the mother). Women who joined and completed the study may have lived in a more positive environment for child growth and development. In this study, over 50% of infants lived with grandparents, and 75% of the mothers reported having a partner (married or common law).

Despite being low-income, participants were not in a dire financial situation. The minimum monthly salary in Mexico, based on the minimum wage of $5.00 USD/day, is $174.00 USD (INEGI 2004). The mean household income reported in this study was 1.2-2.6 times more than the monthly minimum wage ($225.00-450.00 USD). In addition, the participants lived in clean environments as demonstrated by households with sewage disposal and gas stoves (versus wood) for cooking. Thus, income and clean environments were likely contributors to the overall health and positive growth of infants in this study. Pizzaro et al. (1991) also reported adequate growth and low rates of infection in a cohort of Chilean infants who despite being low income, their families had access to health care and lived in clean environments.

5.6. Infant feeding habits

In this cohort, initiation rates of breastfeeding were high (93%). Similarly, Gonzalez-Cossio et al. (2003) reported a 92% breastfeeding initiation rate in Mexico. Nonetheless, very few infants (33%) began breastfeeding 1-2 hours after being born. Though not reported in this thesis, mothers did mention to the researchers that a reason for the delay in initiation of breastfeeding was a perceived inability to produce milk shortly after giving birth (secretion of colostrum).

Because the period of observation ended at six months, the median duration of breastfeeding, 4.5 months, underestimates the true duration of breastfeeding. Therefore, it is unknown how long breastfeeding continued in the second half of infancy. At 6 months, 30% (n=45) of infants were primarily breastfed. Santos-Torres et al. (1990) reported 20.4% EBF rates in Guadalajara at six months. In Mexico, nationally exclusive breastfeeding rates were 20.3% at six months (Gonzales-Cossio et al. 2003). The breastfeeding rates reported in this thesis are higher because breastfeeding was defined differently and less stringently than these other two studies. In Brazil, Victora et al.

(1998) reported that 34% of middle income infants received exclusive or predominant breastfeeding at 6 months.

Infant feeding was not a static practice and varied on a daily basis in this cohort. An ethnographic assessment of Mexican mothers found that infants' feeding regimens are commonly complemented with teas, water, and formula (Morrow et al. 1999). Similarly, mothers in our study routinely complemented breast milk with other liquids or tastes of solid food, and consequently feeding categories used in this thesis were not as homogenous as desired. Categories were derived on frequency of breastfeeds and breastfeeding practices throughout the first four months, and not in the previous day (current feeding method). Frequency of breastfeeds may not be the most adequate marker of intake (WHO Working Group on the Growth Reference Protocol 2002) because frequency provides only an indirect estimation of intake. A more direct estimation of breast milk intake through infant test weighing (weight of an infant taken before and after a breastfeed) is time consuming, and was not feasible for this study. Nonetheless, the study has shown that feeding categories reflected breastfeeding practices. Infants classified as primarily breastfed were breastfed more frequently than the other 3 groups and were more likely to continue primarily breastfeeding to six months of age.

5.7. Growth

There was no evidence of differential growth among the feeding groups at 6 months of age. It is not known if growth differences would appear later in infancy. The literature reports that differences in growth between breastfed and formula fed infants are usually noticeable or significant by 12 months of age (Dewey et al. 1992, Haschke et al. 2000). Eckhard et al. (2001) found no significant differences in weight gain from 0-6 months among the fully breastfed versus not fully breastfed Mexican infants living in semi-rural areas. In contrast, BF infants living in a slum neighborhood of Mexico City demonstrated higher attained weight than FF infants; the difference in attained weight at six months was 800 g (Villalpando and Lopez-Alarcon 2000). One possible reason for these conflicting results may be related to the preparation of infant formula such as type of water, sterilization of bottles and teats, and adequate reconstitution.

Infants in this research study demonstrated positive growth throughout the six month period. Ninety four percent of the infants doubled their birth weight, and only four demonstrated inadequacies in growth (-2SD) at six months. The literature documents that breastfed infants fall below the mean of WHO/NCHS curves at around 3 months and remain below the mean until about 2 years of age (Dewey 1998).On average, our infants were born smaller and lighter than the reference population and remained so until the end of the observation period. In contrast to our findings, Villalpando and Lopez-Alarcon (2001) reported that formula fed infants and not breastfed infants at six months were 1 SD below the mean of WHO/NCHS curves for weight and length, while others have found both breastfed and formula fed infants to remain above the reference mean from 0-6 months (Kramer et al. 2003; Victora et al. 1998). The variability among studies may be of attributed to genetic or environmental differences in infant growth other than feeding practices (Kramer et al. 2003; Victora et al. 1998).

Although many infants in this study had WAZ and LAZ below the mean of the WHO/NCHS reference growth curves, the attained weight (7.4 kg) and length (65.5 cm) of participating infants was comparable to other Mexican infants in research studies. Eckhard et al. (2001) described attained weight and length at six months of 7.21 kg and 64.7 cm, respectively. At six months, Villalpando and Lopez-Alarcon (2000) reported that weight was 7.3 kg and length was 66.7 cm. Thus we feel that infants in the study represented growth that was normal for their country of origin.

Possible reasons for adequate growth performance irrespective of feeding mode may be explained by a physical environment conducive to growth. Infants were born in a Baby Friendly Hospital, had access to health care, were immunized, lived in hygienic environments, attended regular well baby visits throughout the study, and when provided formula, it was adequately and hygienically prepared (de Onis et al. 2004). In more adverse environments (unhygienic, crowding, and poor living conditions) breastfeeding may support faster or better growth (Villalpando and Lopez-Alarcon 2001; Hop et al. 2000).

The regression models presented in this study showed that growth was attributable to biological factors such as maternal height, birth weight, and sex. However, birth weight is influenced by nutrition *in uterô* and maternal height is a consequence of

long-term nutrition status (WHO 1995a). Cohen et al (1995) found that attained weight at 12 months in Honduran infants was related to maternal height. Dewey (1998c) in her review discusses that maternal stature and birth weight explain much of the variability in infant growth among cultures. Maternal stature and birth weight may be proxies for SES status (Dewey 1998c). In our study, household income was not related to growth outcomes perhaps because of low variability in SES status among participants (participants were low-income).

5.8. Infection

Gastrointestinal infections Significant differences were observed in incidence of infection when primarily breastfed were compared to not primarily breastfed infants. Primarily breastfed infants were 1.9 times less likely to have a gastrointestinal infection. The protection granted by breastfeeding was attenuated with formula use. When primarily breastfed and partially breastfed infants were combined into one group *'breastfed'*, the protective effect of breastfeeding was less and the relationship became non-significant. This shows that a minimal dose of breast milk (11 feeds/day) appears to be necessary in order to observe clinically relevant protection against gastrointestinal infections.

Overall, incidence of having at least one GI was low (24%). In comparison, Bhandari et al. (2003) reported a weekly prevalence rate of 28% for diarrhea in Indian infants. Dewey et al. (1995) concluded that BF infants were 2 times less likely to have a GI than FF infants. Pizzaro et al. (1991) reported low rates of morbidity from respiratory and gastrointestinal infections in low-income Chilean infants in similar living conditions as our study participants. Furthermore, participation in the study (timely follow-ups and appropriate treatment of illness) most probably contributed to the prevention and/or fewer recurrences, and/or severity of gastrointestinal illness. Although not reported in this thesis, mothers were knowledgeable in treating GI with oral rehydration solutions and natural home remedies. These reasons may help explain why gastrointestinal illness was not associated with growth outcomes in this study.

In addition, breastfeeding allows for a better management of acute watery diarrhea, dehydration (Brown 2003), and illness induced anorexia (Villalpando and

Lopez-Alarcon 2000), which are common symptoms in gastrointestinal infections. These symptoms if not managed appropriately can lead to severe complications and in some circumstances to death. Also, breastfeeding provides the immune factors to combat an enteric infection, which may reduce the need for antibiotic therapy (Carvalho et al. 2003). These health benefits translate into economic benefits for the household and the health care system (Ball and Bennet 2001). For example, mothers would avoid costly payments for using specialized outpatient services (pediatrician) or, if a more serious case of GI, hospital emergency services (blood analysis, stool samples, oral rehydration solutions or intravenous). Decreased use of hospitals services would contribute to reduced health care costs, because services at the Juan I Menchaca Hospital are subsidized by the government.

Upper respiratory tract infection Although the literature documents inconsistent findings between exclusive breastfeeding and URTI, a plausible protective mechanism may exist due to the exclusivity of breast milk (higher dose of immune factors that may modulate the immune system), and its duration. Infants in this study were not exclusively breastfed and only 30% continued to be primarily breastfed six months, which may partly explain why we found no protective effects of breastfeeding on URTI. However, Kramer et al. (2001) concluded that exclusive breastfeeding to six months did not offer significant protection against upper respiratory infections. Similarly, Rubin et al. (1991) concluded that high intensity breastfeeding did not offer significant protection against URTI. Dewey et al. (1992) did not find differences in URTI rates between BF and FF infants. Lopez-Alarcon et al. (1997) found significantly reduced incidence and duration of respiratory infections in breastfed Mexican infants only from 0-4 months. The high prevalence of respiratory infections in our study population was mainly due to the cold and flu season occurring during follow-up (October- February). Further research is needed to examine if the inverse relationship between breastfeeding and URTI is consistently observed in all populations and socioeconomic groups.

5.9. Iron status

Prevalence of iron deficiency (serum ferritin <12 μg/L) in this population was low (9.7%, n=14) in comparison to other studies in similar populations. Only one infant was classified as having iron-deficiency anemia. At six months, primarily breast-fed infants in our study were four times more likely to be iron deficient than those who were not. Specifically, 20% (n=10) of the primarily breastfed infants were ID at six months. Dewey et al. (1998) reported a similar prevalence of ID in EBF at six months (16.3% had ferritin <12 μg/L); however, her study included LBW infants, which are known to have low iron stores. On the other hand, Pisacane et al. (1995) found Italian infants EBF for longer than seven months were less likely to be ID (22%) at 12 months than those EBF for less than seven months. Exclusive breastfeeding may afford more protection against ID since breast milk provides a source of highly available iron. In exclusive breastfeeding, breast milk is not displaced and consequently not replaced by non-nutritive liquids or beikost low in iron.

The low prevalence of iron deficiency in our study may imply that iron stores of most infants had not been sufficiently depleted at six months. However, continued growth, and inadequate weaning practices (reduced breastfeeding and poor food sources of iron) in the second half of infancy are risk factors for severe depletion of iron stores resulting in the high rates of anemia at 12 months and beyond in Mexican infants (Rivera-Dommarco 2001). Thus it can be inferred that the differences in serum ferritin concentrations among the feeding groups at six months may translate into functional differences (reduced hemoglobin, reduced MCV) that will result in IDA in the second half of infancy and in the second year of life. Therefore, providing information on the determinants of serum ferritin concentrations at six months may be of clinical and public health significance for health practitioners interested in reducing the likelihood of severe depletion of iron stores and IDA beyond six months of age.

The regression model explained 32% of the variability in serum ferritin concentration at six months. Though the variables measured and consequently included in the model are important determinants of serum ferritin concentration at six months, the covariates were unable to explain 68% of the variability. The model's explanatory power may have improved had maternal hematological status during pregnancy been assessed, a

blood sample obtained from the mother at recruitment, or a blood sample obtained from the infant shortly after birth (venous sample or cord blood sample). A study in Mexican infants at nine months of age found that a maternal anemia (Hb <125g/L) detected from 0-6 months post-partum was an independent, significant risk factor for infant anemia (Hb<100 g/L) at nine months (OR= 3.3) irrespective of the duration of exclusive breastfeeding (Meinzen-Derr et al. 2006). Our study was not designed to measure prenatal or postnatal maternal hematological status. At recruitment, infants were assumed to have adequate iron stores because they were born term with a birth weight of 2500g or more. In addition, care was taken to exclude those women who were anemic during pregnancy, or had required blood transfusions during pregnancy. Furthermore, a large number of women (92%) reported to have taken iron supplements during pregnancy. In summary, although the post-natal factors measured in this study determine 32% of the variability in serum ferritin concentration at six months, they remain important factors to consider given the high rates of nutritional anemia due to a low dietary intake of iron in Mexican infants and toddlers.

In this study, determinants of low serum ferritin concentrations at six months were primarily breastfeeding, sex (boys), higher attained weight, and higher household income. Even after controlling for weight at six months, boys remained at risk for lower serum ferritin concentration compared to girls. Domellof et al (2002b) reported similar differences between boys and girls in serum ferritin at 6 months of age. The differences between the genders may result from differences in fetal iron accretion (Domellof et al. 2002b). Also, growth, modeled by attained weight in the first half of infancy, was a risk factor for lower iron stores. In the current study, for every kilogram increase in attained weight at six months there was 1.4 increased odds of lower serum ferritin. The relationship between growth or fast growth and iron deficiency or anemia has been reported by others (Willows et al. 2000; Domellof et al. 2001). Growth leads to a rapid expansion of hemoglobin mass, which depletes iron stores (Fomon et al. 2000). It is not clear why infants living in household with incomes >$260.00 CDN had 1.5 times greater odds of lower serum ferritin. Further research is needed to confirm if this finding is of significance (household income directly influencing iron stores) or if the result was

spurious (household income may be a marker of some variable which our study was not designed to measure).

Although feeding was reported to have a significant negative effect on hemoglobin concentration at six months, very little of the variability was related to the model (R^2=0.06). Mean hemoglobin concentration of our cohort (119 g/L) was higher than what has been reported in other Latin American cohorts. Domellof et al. (2001) reported mean Hb of 110.5 g/L in Honduran infants at six months. In a cohort of low-income Argentine infants, 44% of breastfed infants and 14% of the formula fed infants were anemic (Hb<110g/L) at six months (Calvo et al. 1992); whereas Pisacane et al. (1995) found none of the Italian infants who were EBF for longer than 7 months to have anemia at 12 months. Even when compared by feeding groups, anemia in our breastfed infants (14%) was less than what Dewey et al. (1998) reported in EBF Honduran infants (66%, Hb<110g/L). The different prevalence rates among the studies may be related to differences in infection prevalence, use of iron supplements in pregnancy, and maternal smoking (Domellof et al. 2001).

Based on studies of iron metabolism in adults, it would be expected that iron deficiency precede the development of anemia. However anemia, as reported in our study and by others, was more prevalent than iron deficiency; this suggests that the hemoglobin cut-off used to define anemia overestimates the true prevalence of anemia, or that regulation of Hb concentration in infancy departs from the patterns observed in children or adults (Domellof et al. 2001, 2002a). Because Hb is used to screen healthy infants for IDA in developing countries (WHO 2001a), it is important that Hb be a sensitive marker of iron nutriture. IDA in infants and toddlers is linked to slower motor and cognitive development (Lozoff et al.1996, 2003). The caveat of exclusive breastfeeding to six months is the depletion iron stores in infants born with less than optimal stores, which increases their risk for ID and anemia at six months of age and beyond if iron rich complementary foods are not provided. If the current cut-offs over estimate the prevalence of anemia (low Hb concentration), then Hb <110g/L should not be considered an adequate marker of iron nutriture because functional iron status may still be satisfactory and perhaps not as compromised as initially thought. Using the lower

hemoglobin cut-offs recommended by Domellof et al. (2002a), iron deficiency was more prevalent than anemia in our cohort.

5.10. Trade-offs

"The further development of feeding recommendations requires recognition of the diversity of needs and the evaluation of multiple outcomes such as growth, illness, and micronutrient status..." (Garza and Frongillo 1998 p.816). Thus, it is extremely important to examine the issue of infant health holistically because the trade-offs (costs and benefits) of choosing a particular feeding mode must be weighed. Furthermore, the issue of trade-offs is context specific. The compromises afforded by a feeding mode will most likely differ by country, SES status, and physical environment.

In this study, the trade-off of primarily breastfeeding was increased protection against GI at the cost of lower iron stores (assessed by serum ferritin concentrations at the lower end of the distribution) and increased risk of iron deficiency at six months. Formula-fed infants had more gastrointestinal infections but higher iron stores than primarily breastfed infants. Also, not all primarily BF infants had poor iron status because other factors, in addition to breast feeding, are associated with poorer outcomes in iron nutriture. Furthermore, it may also be speculated that increased consumption of breast milk (Dewey et al. 2001) or the act of breastfeeding, an intense caring behaviour (Frongillo 2000a), may protect against the potential adverse cognitive outcomes associated with ID or IDA. The importance of the aforementioned nutritional or health trade-offs will depend on the relative impact that these compromises have on child survival, health, and development for each society. These compromises will only become apparent in future studies designed to answer these questions.

6. Conclusions

6.1. Main conclusions

In this study, breastfeeding practices were documented as they occurred in a free living cohort of healthy term infants from birth to six months in Guadalajara, Mexico. Although exclusive breastfeeding was not practiced, primarily breastfeeding, as described here, offered significant protection against gastrointestinal infections. Breastfed infants grew adequately, and overall had adequate iron stores; however, because their iron stores were lower than other feeding groups they may be at increased risk for iron deficiency at six months of age and as they grow older.

6.2. Future Research and Policy implications

Future research There is a lack of understanding of metabolic pathways in early infancy. Large scales studies designed to understand possible metabolic pathways (hematology, immunology, and endocrinology) for the protective effects of breast milk on URTI infections, and iron metabolism are needed. Animal models may be helpful in answering some of these specific metabolic questions. As with other health outcomes, culture plays a pivotal role in infant feeding. Qualitative work incorporated into quantitative studies will advance our knowledge of infant health outcomes faster.

Policy implications Healthy child policy should also consider maternal factors and pregnancy outcomes as indispensable determinants of infant health. Our study found growth (weight) to be predicted by maternal height and birth weight. In developing countries, efforts to reduce stunting in women are necessary to help improve growth outcomes in the subsequent generation (Cohen et al.1995). Pregnancies that are conducive to maximal yet healthy fetal weight gain may provide an infant the prenatal advantage for successful post natal growth. Although maternal hematological status was not measured in this study, the prevention of anemia during pregnancy should be considered as a necessary solution to improve the iron status of infants in the first year of life (Meinzen-Derr et al. 2006).

The finding that the provision of sufficient quantities of breast milk, (measured in this study by breastfeeding frequency) in addition to other fluids (teas, water), was

protective against gastrointestinal illness is very positive. The relationship between breast milk and protection against gastrointestinal infections strengthens as exclusivity is reached for two reasons; one, infants do not receive liquids or foods that may be contaminated; and two, infants receive higher doses of immune and other biologically active factors (antibacterial, antiviral, probiotic) known to offer protection against enteric infections. Therefore, had infants in the cohort been EBF, the protection against GI may have been even more evident. Formative research would elucidate if the cultural practice of feeding water and teas is a custom a mother would be willing to relinquish for exclusive breastfeeding. However, given the current cultural practices of infant feeding in Mexico our results emphasize that primarily breastfeeding from 0-6 months confers significant protection against GI. For this reason, primarily breastfeeding should be promoted with clear, consistent messages and endorsed by all health professionals.

Our study found that the prevalence of iron deficiency at 6 months was low. However, Mexican toddlers have high rates of anemia (48%) (Rivera-Dommarco et al. 2001). To reduce the risk of iron deficiency as infants grow older weaning foods rich in iron need to be promoted to all infants at around six months of age. The results of this study also indicate that some infants (primarily breastfed infants, boys, or infants with higher weight gain) may specifically benefit from complementing the diet with iron fortified foods at six months of age or, if feasible, screening for anemia and iron deficiency using serum ferritin and hemoglobin.

The results of this study present the trade-off of feeding choices on infant health. Although breastfed infants are at higher risk for ID when compared to partially breastfed or formula fed infants, this risk can be ameliorated if iron rich foods are introduced at around six months or if iron supplements are provided at this time. The protection against GI in early infancy is a true health benefit of primarily breastfed infants. As was shown in this study, infants who are provided human milk substitutes and displace sufficient quantities of breast milk are at higher risk of a GI in early infancy. Unfortunately, the immunological benefits of breast milk can not be obtained in other foods or vitamin supplements. Therefore, given this scenario it is important that breastfeeding begin the continuum of healthy eating in infancy, and its duration and exclusivity should not be an end-point in itself. In Mexico, breastfeeding should be promoted in conjunction with

timely, safe, and adequate (nutrient rich) complementary foods at around six months of age.

The benefits of breastfeeding are beyond doubt and include immune development (Field 2005), moderating eating habits (Fomon 2001), and neurological development (Dewey et al. 2001); however, health professionals and policymakers should not expect exclusive or predominant breastfeeding to overcome other biological or environmental determinants of infant health (Frongillo and Garza 1998) such as birth weight, gender, maternal height, maternal health status during pregnancy, infant care practices, health care, and living conditions. In addition to breastfeeding promotion, these other factors, if modifiable, must be improved to optimize infant health.

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Appendix 1: Ethics committee certificate

Faculty of Agriculture, Forestry, and Home Economics
Human Research Ethics Board
Approval

is hereby granted to:

Noreen Willows, Growth, health and nutritional status of Mexican infants, 0-6 months of age

for a term of one year, provided there is no change in experimental procedures. Any changes in experimental procedures must be submitted in writing to the HREB.

Granted: September 7, 2004

Naomi Krogman

Naomi Krogman, AFHE REB

Appendix 2: Oral recruitment request

Good morning,

Who has just given birth to their first or second child? How old are you? How much did your child weigh? Do you live in Guadalajara? I ask you these questions because we are following newborn's health during the first six months of life and to enroll them in a study that will follow their growth, feeding habits, and sickness. The study would require you to come to the hospital once a month until the baby's six month birthday. At six months we will take some blood from the baby to check if he/she has anemia. If the baby has anemia, the hospital will provide you with the medication. The pediatric consult and all services would be free of charge. Would you be interested in participating in this study?

Thank you for your time.

Appendix 3: Information sheet

Growth, health, and iron status of infants during the first six months of life according to the method of feeding.

Researcher responsible for the study:
Eva Monterrosa RD

Purpose of the study:

This study would like to look at infant growth, feeding mode, and the health of your child in the first six months of life.

Procedures:

Before you leave the hospital, your baby's birth weight, length and size of his/her head will be measured. Every month, for six months we will ask you to come back to the Juan I Menchaca Hospital. At each monthly visit we will measure your baby's growth. A dietitian will ask you about your baby's nutrition. A pediatrician will see your baby and ask you if your baby is sick that day, the week before or the month previous to your visit.

When the baby is 6 months old a small sample of blood will be drawn from your baby. The blood will be tested to see if the baby needs any iron, dietary supplements or fortified foods.

If you are unable to provide us with your pre-pregnancy weight, weight gain during pregnancy, type of birth, and height, we will obtain that information from your medical chart.

Benefits:

The direct benefit to your baby is a regular monthly follow-up of his/her growth and health by a pediatrician. The blood sample drawn will be used to verify if you child presents iron deficiency or iron deficiency anemia. If your baby is identified to have either iron deficiency or anemia, the hospital will provide iron free of charge.

Risks:

Obtaining blood from your baby is a common procedure routinely performed in infants with minimal risk and generally without complications.

How the information will be used:

The information you provide will help us better understand the nutritional needs of children like yours in Guadalajara. This information will be written in special magazines or presented at special meetings to other people that interested in the results of this study.

Confidentiality:

Your name and your baby's name will only appear in the baseline documents and these documents will be kept confidential. In the analytical phase of the study you will be assigned a code instead of using your or your baby's name.

Time commitment:

The study requires you to come back once a month for 6 months. Each monthly visit will take about 2 hour to complete.

Withdrawal from the study:

You are free to stop participating in the study at any time. If you choose to do so, you will continue to receive all the services offered by the hospital.

If you have any questions please contact:

Eva Monterrosa, Dr. Marta Luna, Dr. Enrique Romero, or Dr. E. Vasquez-Garibay at: 36-18-93-62 ext. 1211 or 36189667

Appendix 4: Consent form

Mother-infant pair No._____
Mother's initials:_____

Growth, health, and iron status of infants during the first six months of life according to the method of feeding.

Researcher responsible for the study:
Eva Monterrosa RD

Do you consent to having you and your baby included in this study about growth and health of infants?
Yes No

Have you received adequate written or verbal information about this study?
Yes No

Do you understand that the risk to your baby is minimal?
Yes No

Have you had an opportunity to ask questions or discuss this study with the researcher?
Yes No

Has confidentiality been adequately explained to you?
Yes No

Have you been informed on how the information you provide will be used and presented?
Yes No

As part of this study, do you consent to have a small sample of blood taken from your baby at his/her six-month birthday?
Yes No

This study was explained to me by: _____

_____ _____
Signature of Mother Date (dd/mm/yyyy)

_____
Printed Name of Mother

Printed Name of Witness	Signature of Witness

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator or Designee	Date

Appendix 5: Baseline questionnaire

1. Mother-infant pair No. ___
2. Mother's Hospital Registry No. _____

CONTACT INFORMATION (Items numbered 3-10)

Name _____
Address _____
Phone Number _____
Date:_____

BABY CHARACTERISTICS

11. Sex of baby: M or F
12. Name of infant and initials: _____
13. Date of birth: _____
14. Type of Delivery: Vaginal ____
    Cesarean ____
15. Reason for Cesarean: _____

16. Weight_____grams
17. Length_____cm
18. Head circumference_____cm

19. When was the child given its first breast-feeding?
    1. Not given
    2. <1 hour after birth
    3. 1-2 hours after birth
    4. 3-5 hours after birth
    5. 6-9 after birth
    6. =10 after birth

MOTHER'S CHARACTERISTICS

20. Age _____ (years)

21. Height_____cm

22. Height reported
    1. self-reported
    2. obtained from medical chart
    3. measured by researcher

23. Pre-pregnancy weight _____kg

24. Pre-pregnancy weight reported:
    1. self-reported
    2. obtained from medical chart

25. Weight gain during pregnancy _____kg

26. Weight gain reported:
    1. self-reported
    2. obtained from medical chart

27. Did you receive iron during your pregnancy? Yes or No
28. Who indicated you to take them?
    1. health care worker
    2. family member
    3. friend
    4. other

29. Did you receive any vitamins during your pregnancy? Yes or No
30. Who indicated you to take them?
    1. health care worker
    2. family member
    3. friend
    4. other

31. During your pregnancy, did you receive prenatal care from a doctor?
    Yes or No

32. If yes, how many times
    1. one time
    2. 2-3 times
    3. 4-5 times
    4. more than 5 times

33. Did you receive information from a nurse or doctor about breastfeeding before you gave birth? Yes or No

34. If yes, please indicate what type(s):
1. Oral
2. Oral with written material
3. Video
4. Written information

35. Did you smoke before you discovered that you were pregnant? Yes or No

36. Did you smoke after you discovered that you were pregnant? Yes or No

37. If yes, how many days as week did you smoke?
    1. 7 days
    2. 6-5 days
    3. 4-3 days
    4. 2-1 days

38. Have you smoked since your child was born? Yes or No

39. Is this your second child? Yes or No

40. If so, how old is your first child? _____ (years)

41. Did you breastfeed your first child? Yes or No

42. If so, how long did you breastfeed your first child?
    1. < 1 month
    2. 1-3 months
    3. 4-6 months
    4. 7-9 months
    5. 10-12 months
    6. >12 months

43. How do you plan to feed this child?
    1. Infant formula
    2. Breastfeeding
    3. A combination of breastfeeding and infant formula
    4. Cow's milk
    5. Combination of breastfeeding and cow's milk
    6. Soy infant formula
    7. other:_____
    8. Don't know

44. If answered #2, #3, or #5 of the previous question, how long do you plan to breastfeed?
    1. < 1 month
    2. 1-3 months
    3. >3 months

45. In the previous month, have you skipped breakfast, lunch or supper because there was not enough food? * Yes or No

SOCIODEMOGRAPHIC DATA

MOTHER:

46. Prior to pregnancy you were:
    1. Full-time employee
    2. Part-time employee
    3. Full-time student
    4. Worked and studied
    5. Unemployed
    6. At home

47. Please specify type of employment
    1. labourer
    2. employee
    3. domestic employee
    4. merchant
    5. professional
    6. other: _____

48. If employed or going to school prior to pregnancy, do you plan to return to work or school? Yes or No

49. If yes, please specify when.
    1. <45 days
    2. 45-90 days
    3. 91-120 days
    4. 121-180 days
    5. >180 days

50. How many years of education have you completed?
    1. no education
    2. can read and write
    3. elementary school not completed
    4. elementary school completed
    5. junior high not completed
    6. junior high completed
    7. junior high with a technical route
    8. high school not completed
    9. high school completed
    10. post-secondary education

51. What is your marital status?
    1. single
    2. common law
    3. married
    4. separated
    5. widowed
    6. divorced

FATHER OF THE CHILD

52. How old is the child's father? _____(years)

53. What does the father of your child do for a living?
    1. Full-time employee
    2. Part-time employee
    3. Full–time student
    4. Works and studies
    5. Not a wage earner
    6. Don't know

54. Please specify type of employment
    1. labourer
    2. chauffer
    3. employee
    4. mechanic
    5. merchant
    6. professional
    7. other:_____

55. How many years of education has he completed?
    1. no education
    2. can read and write
    3. elementary school not completed
    4. elementary school completed
    5. junior high not completed
    6. junior high completed
    7. junior high with a technical route
    8. high school not completed
    9. high school completed
    10. Post-secondary education
    11. Don't know

56. What is the monthly income of your household? (pesos)
    1. <1240
    2. 1240-2480
    3. 2481-4960
    4. >4960

57. How much do you spend on food per month? (pesos)
    1. <500
    2. 500-1000
    3. 1001-1500
    4. 1500-2000
    5. >2000

HOUSING CHARACTERISTICS

58. Is the dwelling where you live?
    1. Owned by you or your partner
    2. Rented
    3. Borrowed
    4. I live with my parents or in-laws

59. How many people live in the house? _____

60. How many are children?_____ (<16 years old)

61. How many bedrooms are in your house?
    1. one
    2. two
    3. three
    4. four
    5. more than four

62. What type of sewage disposal do you have for your home?
    1. Sewage system
    2. Latrine
    3. other: _____

63. What type of energy do you use for cooking?
    1. gas
    2. wood
    3. electricity
    4. coal
    5. other: _____

64. Do you have a refrigerator to store food? Yes or No

Appendix 6: Monthly questionnaire

IDENTIFICATION

1. Mother-infant pair No._____
2. Visit number: _____
3. Infant hospital registry No._____
4. Mother's initials _____
5. Age of infant (days) _____
6. Date of Birth _____

7. Sex: M or F
8. Today's date _____

ANTHROPOMETRY

9.Weight:_____g

10. Length: _____cm

11. Head circumference: _____cm

FOOD

12. How are you feeding your baby?
    1. breast milk
    2. formula
    3. combination of breast milk and formula
    4. whole cow's milk
    5. combination of breast milk and whole cow's milk
    6. other: specify_____

13. In the last month did you feed your baby ONLY breast milk? Yes or No
    Clarification, if needed: no other liquids or solids other than breast milk, except vitamins and minerals indicated by a doctor

14. If no, please tell me how many times in the last month you gave your baby:
    Water: _____ daily/ weekly/ monthly
    Teas: _____ daily/ weekly/ monthly
    Juices: _____ daily/ weekly/ monthly
    Breast milk: _____ daily/ weekly/ monthly
    Formula: _____ daily/ weekly/ monthly

Other milks: _____ daily/ weekly/ monthly
Fruit: _____ daily/ weekly/ monthly
Solid or Semi Solid: _____ daily/ weekly/ monthly

15. In the last month, have you begun to wean your baby from the breast? Yes or No

16. If yes, please indicate the reason why you initiated weaning _____

17. If you are feeding your baby formula or powdered milk, please indicate the name of the product you are using? _____

18. How do you dilute the formula or powdered milk?

1. measuring scoop per ounce of water
2. measuring scoop per two ounces of water
3. measuring scoop per three ounces of water
4. measuring scoop per four ounces of water

19. What type of water do you use to prepare the formula or powdered milk?

1. boiled water
2. bottled water
3. boiled, bottled water
4. tap water

20. Do you boil the bottle and nipples? Yes or No

21. Does you baby receive vitamins? Yes or No

22. If so, how many drops? _____

23. With what frequency does the baby receive these drops?

1. daily
2. weekly
3. monthly

24. Indicate the name of the vitamins that your baby receives: _____

25. Does your baby receive iron? Yes or No

26. If so, how many drops? _____

27. With what frequency does your baby receive the drops?

1. daily
2. weekly
3. monthly

28. Please indicate the name of the iron that your baby receives: _____

29. In the previous month, did someone give you advice on how to feed your baby?

Yes or No

30. If yes, who advised you?

1. friend
2. family member
3. health care worker

31. If yes, please indicate what the main message you understood was.

24 hour recall. Please ask if the infant receives any other liquids such as teas, water or if anything has been added to the bottle such as sugar, honey or cereals.

PEDIATRIC ASSESSMENT

32. Temperature: _____

33. Today does the infant have any of the following symptoms?

___a. runny nose
___b. cough
___c. fast breathing
___d. increased stool frequency
___e. loose stools
___f. vomiting
___g. fever

ILLNESSES

34. In the previous month, did your baby have any of the following symptoms?

Please indicate duration also

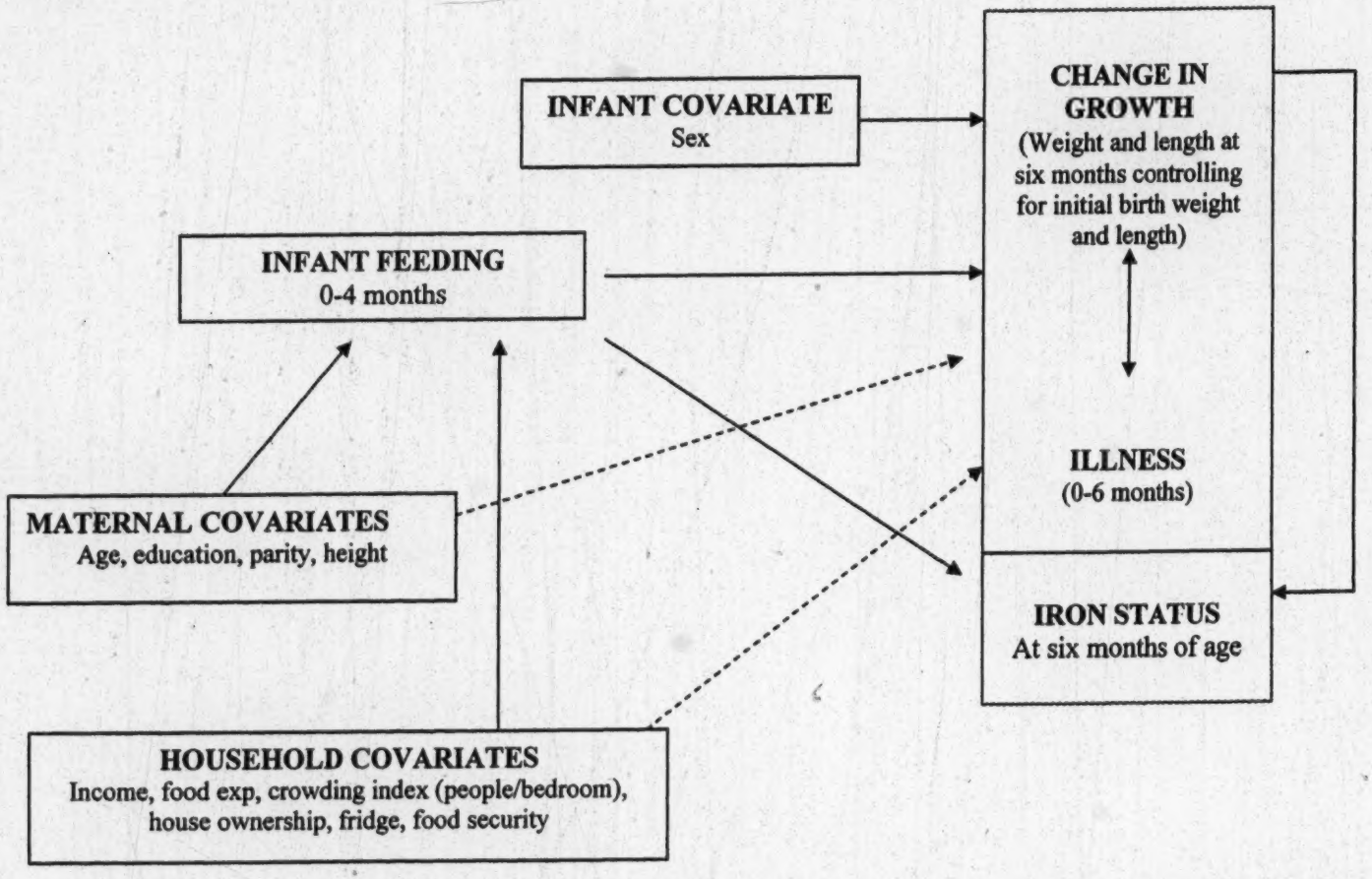
___a. runny nose
___b. cough
___c. fast breathing
___d. increased stool frequency
___e. loose stools
___f. vomiting
___g. fever

35. Because of these symptoms, did you take your baby to the doctor?
Yes or No
36. Was a diagnosis given? Yes or No
37. If yes, what was the diagnosis? _____
38. Was your baby hospitalized because of the diagnosis?
39. Is the infant up-to-date with the vaccinations?

QUESTIONS FOR THE MOTHER

40. Have you returned to work or study in the past month? Yes or No
41. Do you smoke? Yes or No
42. If so, how many times a week do you smoke?
    1. 7 days
    2. 6-5 days
    3. 4-3 days
    4. 2-1 days
43. Does the baby attend daycare? Yes or No
44. If so, what time does the baby attend daycare?
    1. morning
    2. afternoon
45. How many hours does the baby attend daycare? _____
46. Aside from daycare, does another person take care of your baby?
    1. family
    2. friend

Appendix 7: Conceptual model



Appendix 8: Coding for analyses

Infant feeding Dummy variable

	X1	X2	
Full	1	0	
Partial	0	1	
Formula	0	0	(control group)

Label	Code: 0	Code:1
Education (categorical-ordinal)	Completed elementary education	More than elementary education
Parity (dichotomous)	primiparous	not primiparous (second child)
Children in the home (ordinal)	No children	One or more
Food insecurity (dichotomous)	Not insecure	Insecure
House ownership (categorical-nominal)	Own home	Not home owner
Refrigerator in home (dichotomous)	Yes	No
Monthly Income (categorical-ordinal)	= 2 minimum salaries	>2 minimum salaries
Feeding2categories (categorical-nominal)	Primarily breastfed	Not primarily breastfed (Partially breastfed and formula fed combined)
Formula feeding 2 categories (categorical-nominal)	Not formula fed (primarily breastfed and partially breast fed combined	Formula fed
Sex of infant (dichotomous)	Girls	boys
Birth weight (continuous)	1: 2500-3000 2: 3001-3500 3:3501-4000	This variable not coded as a dummy

Appendix 8: Coding for analyses

Infant feeding Dummy variable

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Formula feeding 2 categories (categorical-nominal)	Not formula fed (primarily breastfed and partially breast fed combined	Formula fed
Sex of infant (dichotomous)	Girls	boys
Birth weight (continuous)	1: 2500-3000 2: 3001-3500 3:3501-4000	This variable not coded as a dummy